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PRINCIPLES

OF

BIOCHEMISTRY

FOR STUDENTS OF MEDICINE, AGRICULTURE AND RELATED SCIENCES

BY

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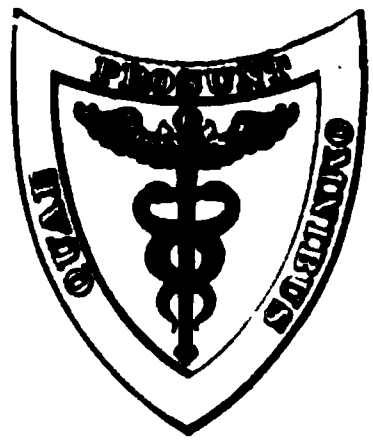

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DEDICATED

TO THE MEMORY OF MY FRIEND AND TEACHER

SIR EDWARD C. STIRLING

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IN TOKEN OF INTELLECTUAL INDEBTEDNESS AND

PERSONAL AFFECTION

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PREFACE.

It has been the object of the author, in writing this book, to present the subject of Biochemistry in close relationship to Physiology, so that the student may perceive the intimate dependence of these two sciences upon one another and come to regard physiological chemistry in its true light, as the foundation upon which we must ultimately build our interpretations of the *functions* of living matter. Emphasis has been placed upon the practical applications of the subject, and not only upon applications to the practice of medicine, but also upon applications to the industries and to general biology, for while the design of the author has been primarily to write a text-book for the use of medical students and students intending to specialize in biochemistry and physiology, the attempt has also been made to compile a work which will be of service to the agricultural student, the student of general biology, or the industrial chemist who is engaged in handling biological products.

I am deeply indebted to my colleague, Prof. Hardolph Wasteneys, for his valuable coöperation in preparing the manuscript for the press, correcting proofs and compiling the index; and I desire to acknowledge my indebtedness to my wife for her assistance in the preparation of some of the illustrations.

T. Brailsford Robertson.

Adelaide, South Australia, 1920.

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PRINCIPLES OF BIOCHEMISTRY.

INTRODUCTION.

THE NATURE AND SCOPE OF THE SUBJECT.

The subject-matter of biochemistry is the application of the known principles of chemistry and physical chemistry to the study and interpretation of life-phenomena; of the processes, that is of digestion, assimilation, respiration, growth, reproduction, muscular contraction and the like, which combine to distinguish living from inanimate matter. From this definition it must be clear that biochemistry possesses very close affiliations with both animal and vegetable physiology. For physiology is the study of the way in which societies, individuals, organs and cells perform their functions, and since each and every function of living matter ultimately involves or depends upon chemical changes, to this extent the study of each and every function of living matter becomes a part of the subject-matter of biochemistry.

The distinction between physiology and biochemistry is in fact an arbitrary one, depending very largely upon convenience and upon the contemporary limitations of our knowledge.

So long as we possess no clue whatever to the nature of the processes which underlie or accompany a life-phenomenon, the study of that phenomenon and of the method of its performance is, beyond any question, an exclusively physiological problem. But directly we take the first steps toward ascertaining the nature of the *chemical* phenomena which accompany its performance, we are taking, also, the first steps toward incorporating this problem into the subject of biochemistry.

The historical growth and development of the subject have illustrated very aptly these natural applications. In the beginning, and that only one brief generation ago, biochemistry was an undifferentiated portion, a minor branch of physiology, and formed the subject of a bare half-dozen lectures delivered by the professor of physiology. Gradually the need of special training for the study of this subject, and its continually increasing magnitude and practical importance have led men to make a special study of it, apart from that of the parent-subject. The labors of these men have quickly added countless phenomena to their special domain, and so important are these, and so fundamental is the part which biochemistry now plays in medicine, agri-

culture and the industries, that almost everywhere the study of biochemistry ranks with that of anatomy, physiology and pathology as one of the studies fundamental to the understanding of medical science, or with botany, plant-physiology and bacteriology as one of the studies fundamental to the understanding of agriculture.

It must not be supposed, however, that the withdrawal of biochemistry from the parent-subject has left physiology any the poorer. Physiology has not been left merely with a residuum of undigested material, ultimately to be absorbed by the biological chemist. On the contrary, with the development of biochemistry, physiology has developed too and that to an extent unimagined by its founders. A few generations ago, physiology was a little-considered fragment of the study of anatomy, just as, one generation ago, biochemistry was a little-considered portion of the study of physiology. The same differentiation has separated the teaching of biochemistry from physiology as that which has separated the teaching of chemistry from that of physics. We may regard physiology as consisting for the present of the study of the applications of anatomy and physics to the elucidation of life-phenomena, together with the entire study of a residuum of phenomena and processes which are for the present passed by in biochemistry simply because we do not as yet possess any clue whatever to the nature of the chemical processes which underlie them.

Hence, physiology is destined ultimately and at some as yet far distant date to become the study of the interpretation of life-phenomena by the aid of the principles of anatomy, gross and minute, and physics. Biochemistry is the study of the interpretation of life-phenomena by the aid of the principles and facts of chemistry. Physiology investigates the molar and molecular phenomena of life, biochemistry the atomic.

Of course, this division is arbitrary and unreal, just as the distinction between physics and chemistry is arbitrary and unreal. Nature recognizes no such classification of her phenomena. Physics merges insensibly into chemistry and in like manner physiology merges into biochemistry. An illustration of this fact has been strikingly afforded in recent times by the rapid development of physical chemistry, a whole borderland between physics and chemistry, which has undergone such extensive survey within the last generation as to demand a noteworthy degree of special training on the part of those who would attempt to master it. Even the delineation of this domain has not by any means removed all of the "debatable land," however, that lies between physics and chemistry; witness the recently discovered phenomena of radio-activity, which have opened up yet another field of investigation which is neither physics nor chemistry. And so it is with physiology and biochemistry. There is an indefinite "debatable area" between the two, and many if not most of the problems in either field require the aid of both physiology and biochemistry for their solution.

The investigator of Nature cannot afford to hamper himself by arbitrary definitions and delimitations of his field. When the need arises he must be prepared to use the tools which the problem calls for, be they the tools of physics, chemistry, mathematics, anatomy, bacteriology or pathology. The teacher is somewhat more constrained. He cannot carry his pupils too far from the center of the subject in hand, lest their lack of preparation should render them unable to follow him. Even so, however, the student of biochemistry will often have occasion to dwell, in his studies, upon certain aspects of problems which the physiologist has made peculiarly his own, and the medical student will frequently find himself studying one and the same problem in his course in biochemistry and again in his course in physiology. Nevertheless he will find that he is not merely repeating his work, not merely covering old ground, but that, on the contrary, the physiologist and the biochemist have each of them something different to say; displaying the problem in different lights and dwelling upon it in different connections.

We have stated that the science of biochemistry consists in the interpretation of life-phenomena in the light of the facts and principles of chemistry. The question may here very naturally arise in the mind of the reader, How can it be possible to apply chemistry to the investigation of living matter? True, we can attempt to analyze living matter, to separate chemical constituents from it and to identify them. But then, directly we begin to analyze living matter it ceases to be living matter. The reagents which we employ immediately "kill" it, that is to say, abruptly suspend its characteristic functions and disperse and dissolve the minute structures of protoplasm which are the physical substratum upon which its functional activities are reared. Unquestionably, an *amæba* which has been boiled in hydrochloric acid may yield interesting products, but then it is no longer an *amæba*, and the products which analysis yields bear only a remote relationship to those which were originally present in the living organism.

To find out what is actually occurring in *living* matter we must, therefore, employ methods of investigation somewhat analogous to those which the physical chemist employs in the investigation of what is actually occurring in flames. First, we study the nature of the substances which enter the flame, then we study the properties and behavior of the flame itself, always taking care to do so by the aid of instruments which do not disturb the flame, and finally we ascertain what substances the flame gives off. From these various and fragmentary data we endeavor to reconstruct in our minds a coherent picture of the train of events as they actually occur, and this endeavor will be the more successful in proportion to the extent, the variety and exactitude of our measurements.

So far as possible, then, we must bring *static* and not *dynamic* methods of mensuration to the study of living matter, methods, that is, which do not involve the cessation of the very processes which we desire to

investigate. Often, it is true, we can successfully employ destructive, dynamic methods to find out many important things. For example, in the study of digestion, we can destroy the living cells which form the lining mucous membrane of the stomach, and, having destroyed them, extract from them a substance, *Pepsin*, which will digest proteins, even in glass vessels in laboratory-incubators. In this way and by similar methods we can study the changes which are brought about in our foodstuffs when they enter the alimentary canal. Even in a fairly simple case such as this, however, the dynamic method does not altogether suffice. For we find that within the alimentary canal itself the foods are digested much more rapidly than we can digest them with the aid of ferments in laboratory-glassware. Some condition, other than mere warmth or mechanical agitation, some condition which we have not yet fully succeeded in imitating, very materially aids the action of these ferments in the cavity of the living alimentary canal.

By such phenomena as these we are constantly being reminded that it is not by any means safe to argue directly from the behavior of dead fragments or products of living tissue, to that of living tissue itself. The results of dynamic experiments which involve the actual destruction of the living tissues which we are investigating, only afford a starting-point, therefore, or an orientation, for our guidance in a repetition of the experiment under actual living-conditions.

Biochemistry, therefore, falls very naturally into two fields of study, differentiated by the methods of investigation employed. The one field, that which has until recently been the peculiar interest of the "physiological chemists," consists in the study of the crude substances which enter into the life-flame and the products which leave it. The foodstuffs and the excreta, and, incidentally, the composition of dead matter that once was living, also the study of the action and reaction of fragments of living or dead protoplasm upon the foods or upon one another, these, until comparatively recently, comprised the whole activity and interest of chemistry in the investigation of living matter. It is obvious, however, that while knowledge of these things is an essential prerequisite to the understanding of the chemical phenomena of life, yet they are far from yielding information as to the nature of life-processes themselves. It was for this reason, and with justice, that one of the greatest contributors to our knowledge in this field, G. von Bunge, exclaimed in 1894, "All processes in the organism which may be explained mechanically are no more phenomena of life than are the movements of the leaves and branches of a tree that is shaken by the storm, or the movement of the pollen that the wind wafts from the male poplar to the female."[1] We were at that time hovering upon the outskirts of the main problems, since actual penetration of them was necessarily deferred until the momentous advances of physical chemis-

[1] Lehrbuch der Physiologischen und Pathologischen Chemie, 3te Aufl., Leipzig, 1894.

try placed in our hands the necessary implements and knowledge to essay the task.

Our second field of study, then, consists in an analysis of the chemical phenomena which accompany or underlie the activities of living, undisturbed, and more or less normally functioning protoplasm, a field which until recently was almost exclusively the preoccupation of the "experimental biologist." Inevitably, however, these two phases of chemical inquiry, so closely affiliated, so mutually dependent, are coming to rely more and more intimately upon each other and hence are being welded more and more firmly into one. Experimental biology drawing upon the rich resources of physiological chemistry, is immensely increasing its exactitude and its certainty, while physiological chemistry, on the other hand, is rapidly widening the horizon of its inquiries in response to inspiration drawn from the field of experimental biology. In this work we will recognize no distinction between these fields, but endeavor, in so far as the limitations of our knowledge permit, to interweave them into one coherent representation of the complex tissue of chemical processes which constitutes life and its immediate consequences.

THE DEGREE OF EXACTITUDE ATTAINABLE IN BIOCHEMISTRY.

In the so-called "exact sciences," to wit, mechanics and physics, we have, as a rule, the power to isolate more or less completely any phenomenon or group of phenomena which we wish to study, and to guard them from disturbance by the intrusion of accidental variables. For example, it is not a difficult matter to demonstrate that a falling body experiences a constant acceleration, the most serious intrusive variable being the friction of the air, a variable which can now be very readily excluded in a variety of ways.[1] Similarly, in chemistry, it is not a difficult matter to observe the progress and equilibrium of such a reaction, as, for example, the reduction of iron oxide by hydrogen. The chemicals are procurable in pure conditions, only one reaction occurs, and it is a simple matter to exclude other chemicals and to keep the temperature and pressure of the system constant. In organic chemistry much more complex phenomena are encountered. It is the exception rather than the rule to find a reaction which proceeds evenly and without disturbance by side-reactions or secondary decompositions. To detect regularities and establish "laws" in such a system is a task the more complex the greater the number of adventitious variables.

The difficulties which are encountered in studying organic reactions in laboratory glassware are enormously magnified in studying reactions

[1] It must be remembered that the friction of the air, which to us presents no difficulty, was to our ancestors an insuperable obstacle to the measurement of gravity. In exactly the same way insurmountable obstacles which at this day defeat our ends in physiological or biochemical research will appear of trivial importance to our intellectual heirs. As a rule such obstacles merely imply that we are attacking the problem from the wrong angle.

which occur in living matter. The life of any one cell consists in a multiplicity of parallel reactions, interrelated, interdependent, and interwoven into a bewildering complex. Multicellular organisms, such as ourselves, consist of millions of such cells. When the reader is reminded that the reactions in each organ or group of cells and possibly, even in each individual cell, possess an individual character of their own, and that these reactions are excessively sensitive to external agencies, the complexity of the task of unravelling the separate reactions and tracing their individual progress must be evident.

It follows from the complexity of the phenomena that the regularities and relations observed by the biochemist are rarely capable of formulation with such precision as those which are observed by the physicist or chemist. To illustrate this fact, let us consider the difficulties attendant upon the investigation of one of our simplest problems, to wit, that of the mode of action of the protein-digesting ferment **Trypsin.** We have first of all to overcome the difficulty of obtaining pure protein. That obtained (and a "pure" protein in the sense that inorganic reagents may be "pure" has never been prepared), we then have the difficulty of obtaining a pure trypsin, a difficulty which has never been even partially overcome. In fact we certainly possess no pure trypsin and we have, moreover, no method of ascertaining how impure our preparations are. Not only are our preparations of trypsin impure, but they frequently contain several ferments which digest proteins, a fact which has only recently come to be appreciated. Notwithstanding all these obstacles we have found that if trypsin be allowed to act upon protein, with certain necessary precautions, a regularity may be observed in the rate of decomposition of the protein by the ferment, and this regularity may even be formulated in mathematical terms. We are not surprised to find, however, that the agreement between the formula and the experimental measurements (of quantity of protein digested) is not extremely exact. Under very favorable conditions the requirements of theory and the findings of the investigator may agree to within one per cent. of their mean value. In a purely chemical problem an agreement to within one-tenth of a per cent. is anticipated and not infrequently obtained. In physics or in astronomy an agreement to within one one-hundredth of a per cent. is not in the least exceptional. As the uncontrollable adventitious variables become fewer, it will be observed, the agreement between formulæ and experimental data becomes more and more precise.

Hypotheses of a more general character, not admitting of mathematical formulation, share in this disadvantage, and hence it arises that a larger proportion of hypotheses in biological sciences are of uncertain or very questionable validity than of those in the so-called "exact" sciences. But the difference is merely a matter of degree and tends progressively to diminish. All scientific hypotheses and "laws" are subject to a marginal inexactitude, and all human precision is relative. As our acquaintance with any field of investigation grows

more extensive, the width of the margin of inexactitude diminishes, and that is all. For example, no "law" is apparently of more extensive applicability or capable of more precise mathematical formulation than Newton's law, that bodies attract one another as the inverse square of their distance apart. Yet certain astronomical data, deviations in the orbit of the planet Mercury,[1] point to the possibility that even this law may not be exact and that the true exponent of the distance may in truth not be 2 but 2.000,0001612. The margin of inexactitude is here represented by the minute fraction 0.0000001612, but it is here nevertheless. And so it is with all scientific hypotheses. Our laws, formulations and hypotheses are merely temporary shorthand statements of our acquaintance with the facts. As our acquaintance with the facts grows larger we must revise our shorthand to express our accessions of knowledge. The shorthand is not the knowledge itself. Science, in reality, consists solely in our knowledge of facts and our control of the forces of nature and not of the hypotheses which we formulate by the way in order to summarize our present state of knowledge and stimulate the imagination to fresh inquiries. Biology is, in truth, no less an "exact" science than any other, than astronomy, for example, but its *hypotheses* are subject to much more frequent and thorough revision than those of physics or astronomy, simply because our knowledge of the field is less and is growing more rapidly.

The whole theory of the scientific method of thought has in fact been based by the great founders of science upon the assumption of the fallibility of purely intellectual operations, and hence of the untrustworthiness of hypotheses. Newton's famous rule, "*Hypotheses non fingo,*" while impracticable for the individual investigator, remains nevertheless true of science as a whole, of the body of exact knowledge, that is, which endures the test of time and endows mankind with the power of ruling and directing the multifarious and stupendous forces of Nature. During the centuries which have been marked by the acquirement of this knowledge countless hypotheses have been formed, and accepted for a while, and then abandoned as evidently absurd. But the forward march of exact knowledge has never suffered interruption and not infrequently indeed has been very much facilitated by the most obviously erroneous hypotheses. The phlogiston theory of heat is perhaps the most striking example of this kind. It was a most patently erroneous hypothesis, built up by perfectly sound reasoning based upon imperfectly understood facts. Yet for a hundred years the mere existence of this hypothesis was the greatest contemporary stimulus to the development of chemistry and it ultimately led to the establishment of the conception of the conservation of matter.

As the curves of the geometrician approach and yet never actually attain their asymptote, so do we continually approach and never yet have we attained the utter truth. The merit of the scientific method

[1] Cf. article on Gravitation, Encyclopedia Britannica, 11th edition.

of thought lies in the fact that the otherwise circular speculations of humanity, ever returning unprofitably to the point from which they started, have had a thrust communicated to them which has deflected them into a perpetually widening spiral, reaching further and ever further into the infinite, promising knowledge commensurate only with the immensity of the universe, and power to which no man dare set a limit.

If, then, our present conceptions in biochemistry are subject to rapid and comprehensive modification this affords no legitimate basis for scientific cynicism or indiscriminate scepticism. On the contrary it is a hopeful augury, testifying to the youth of the subject and the vast development that lies before it. No subject, indeed, promises more immediate developments of stupendous significance to man. The control of life itself, no less, is the alluring aim and destiny of the medical and biological sciences and the basis of every step in the acquirement of this control must inevitably be founded on a knowledge of the chemical processes which underlie and constitute life. We may be well content, with such a prospect before us, to resign absolute certainty to the political doctrinaire. For ourselves, dwelling amid uncertainties and hazards, advancing like bold navigators in uncharted seas, we will turn our faces toward the new and wider horizons which always lie before us. We will regard a hypothesis as an instrument of research, like a balance, a burette, or better still a compass; a guide and a stimulus to investigations, but a mere approximation to the truth which we trust will gradually approach closer and yet closer to verity as our knowledge grows in extent and proliferates in detail.

THE PREPARATION REQUIRED FOR THE STUDY OF BIOCHEMISTRY.

No amount of courage and enthusiasm, however, will suffice to altogether compensate for lack of preliminary training and acquired skill in those branches of science upon which biochemistry is founded and from which it originates. Biochemistry is in the first place and most essentially an outgrowth from organic chemistry and an acquaintance with the general principles of that science and the simpler laboratory procedures most frequently employed in it, is as essential to the understanding of biochemistry as a vocabulary of French words is to the understanding of Molière in the original. In this work I will suppose the reader to be acquainted with organic structural formulæ and the general principles according to which they are inferred from the behavior of the substances to which they are applied.[1]

The modern developments of biochemistry and particularly those which aim at the interpretation of the processes underlying the performance of function, involve the application of the elementary princi-

[1] The reader whose previous training in this subject has been deficient may consult E. V. McCollum, Organic Chemistry for Students of Medicine, New York, 1916.

ples of physical chemistry and there can be no doubt whatever that the future and most momentous developments of the subject are destined to involve physical chemistry more and more extensively. The essential principles are neither numerous nor abstruse, good elementary text-books of the subject abound and the student is earnestly recommended, if he has not previously received training in this subject, to acquire for himself a suitable handbook of physical chemistry,[1] and to consult it frequently in the course of his studies in biochemistry.

The intelligent employment of the elementary principles of physical chemistry implies a nodding acquaintance with the so-called "higher mathematics," but far more than for the mere understanding of physical chemistry, mathematics is an essential instrument in the handling of quantitative measurements of any kind whatsoever. Every branch of science is, in its youth, qualitative, and in its maturity quantitative. Even taxonomy has been converted by the discoveries of Mendel into a quantitative study involving in some instances very complex mathematical operations. Biochemistry is at the present juncture passing through a species of adolescence and emerging by very rapid stages from the qualitative into the quantitative stage of development. The student who would prepare himself for the future, therefore, would do well to acquire such rudiments of mathematical skill as may be necessary for the elucidation of principles which he will unquestionably be called upon to comprehend. Mathematics is in reality a symbolic language which expresses in brief terms a series of interrelated facts and considerations which would otherwise be too intolerably complex to retain simultaneously in the mind. By acquiring mathematical facility, therefore, the student is not augmenting the complexity of his task, but simplifying it.

The applications of physical chemistry to biological problems necessitate of course an elementary knowledge of the differential calculus and the simplest methods of integration.[2] All of the work upon ferments and digestion in its quantitative and most important aspects now demands the employment of the calculus. As an example of the wider and at first sight unexpected applications of this mathematical technique the reader is referred to the important work of Barcroft,[3] which has marked an epoch in our understanding of the respiratory functions of the blood and which could never have yielded one tithe of the information obtained without the employment of the methods of the differential and integral calculus.

A moderate familiarity with the elementary principles involved in the solution of differential equations would also upon occasion be found

[1] For example, E. W. Washburn: An Introduction to the Principles of Physical Chemistry, New York, 1915. A. Findlay: Practical Physical Chemistry, London, 1914.

[2] The student may consult J. Edwards: Differential Calculus for Beginners and Integral Calculus for Beginners, while for the methods of applying the calculus to the solution of scientific problems the student would do well to read Perry's Calculus for Engineers, London, 1897.

[3] The Respiratory Function of the Blood, Cambridge, 1914.

very useful.[1] In the treatment of quantitative data and the graphic representations thereof it is frequently necessary or desirable to apply a formula to the curves obtained or to compare them with the curve which may be deduced from theoretical premises. In this extensive field of practice a knowledge of the proper method of dealing with and minimizing the effect of accidental experimental errors is required and the employment of the method of least squares is essential if the best use is to be made of the experimental material which may be available.[2] For purposes of fitting empirical formulæ to curves, eliminating excessively erroneous results and interpolating probable values between values which have actually been measured, a study of the methods of interpolation and mechanical differentiation is exceedingly valuable and helpful.[3]

But perhaps the most essential branch of mathematical practice in the equipment of the biochemist of the future will consist in the methods of the statistician. When we come to deal with actually living material, as we are compelled to do in order to advance our subject at all in its most significant direction, we are at once confronted by the problem created by the inherent *variability* of living things. No two animals are alike, not even may we find any two living cells which are precisely identical. In agriculture no two plots of ground are alike, no two plants are ever identical. How then, in comparing experimental animals or plants or plots of ground with "normals" or "controls" shall we ever attain to certainty of our results? It would seem that it must always be possible that the differences between any two groups of animals may merely be the product of chance selection of two groups which might have differed in the observed sense without any experimental manipulation whatsoever. This difficulty, the fundamental character of which is recognized by every biological investigator, is of course not of so much importance in those cases in which the differences for which we are looking are very large, as death contrasted with survival, decisive loss of weight contrasted with equally decisive gain, or reduction or enhancement of normal qualities by fifty per cent. or more. But phenomena such as these are the obvious ones in any field of science, those which lie at the surface and are garnered by the earliest investigators, and they are not invariably, and in fact not usually, the phenomena upon which we ultimately come to rely for the basis of wide and fundamental generalizations. Such emphatic disparities testify in themselves to the unusualness of the conditions invoked, and hence carry the suspicion that the response to such extreme conditions may not be a normal or at least a usual reaction of living matter to its environment. For our deeper

[1] Murray: Introductory Course in Differential Equations, London, 1897.

[2] M. Merriman: Text-book on the Method of Least Squares, New York, 1891. L. Tuttle: The Theory of Measurement, Philadelphia, 1916.

[3] H. L. Rice: The Theory and Practice of Interpolation, Lynn, Mass., 1899. J. Mellor: Higher Mathematics for Students of Chemistry and Physics, London, 1902.

understanding of life which is to come therefore, we must learn to rely with confidence upon relatively small and fluctuating disparities between groups composed of very variable material. This can be done in one way and in one way only, namely, by employing the methods of the statistician whereby we may accurately gauge the relative values of observations obtained with variable material, compute the number of observations necessary to attain a given degree of certainty or accuracy, place in their proper perspective extreme or overlapping variations in aberrant individuals and, in short, render measurements upon even such variable material as living organisms just as precise as the measurements employed in quantitative analysis.

The student of biochemistry would be well-advised therefore to acquire the simple mathematical technique which is requisite for the employment of statistical methods,[1] but he should remember that this branch of mathematics above all others abounds in pitfalls for the unwary and he should be sure that he perfectly comprehends the simple fundamental principles which underlie these methods before he attempts to put them into practice.[2] If the reader should desire to gain a conception of the variety and scope of the possible applications of the statistical method to problems of biochemistry, experimental biology and agriculture, he may consult the recent work of Loeb and Wasteneys upon the applicability of the Bunsen-Roscoe law to animal heliotropism,[3] of Waynick upon the distribution of nitrifying bacteria in soils[4] and of the author upon the growth of children.[5]

The Subdivisions of the Subject.—In this work we will endeavor to follow up the foodstuffs from the moment when they are partaken of, to the moment when, after having circulated through the body and partaken of its life, their final products are excreted. The subject-matter is divided into six parts corresponding with various phases of the cycle of changes which the foodstuffs undergo. The subdivisions are as follows:

Part I.—The Foods, their properties, digestion, assimilation and conversion into living matter or into reserve-materials. The considera-

tion of this phase of our subject takes us up to the point at which the foodstuffs, subjected to certain modifications, have really been converted into living protoplasm. This leads us naturally to the consideration of the second phase of our subject, namely:

[1] The Student may consult G. Udney Yule: An Introduction to the Theory of Statistics, London, 1911. For tables and formulæ the student may refer to C. B. Davenport: Statistical Methods, New York, 1904.

[2] Probably the best introduction to the fundamental conceptions of probability which form the basis of the statistical method is contained in the classical little memoir of W. A. Whitworth entitled Choice and Chance, Cambridge, 1901.

[3] Jour. Exper. Zoöl., 1917, **22**, 187.

[4] D. D. Waynick: University of California Publications in Agricultural Sciences, 1918, **3**, 243.

[5] T. Brailsford Robertson: Am. Jour. Physiol., 1915, **37**, 1 and 74; 1916, **41**, 535, and 547.

Part II.—The manner in which the properties of the foodstuffs mould and determine the properties of living protoplasm.

Part III.—In proceeding to consider the *activities*, apart from the merely passive *properties* of living matter, we are at once confronted with the significant fact that the multicellular organisms, like ourselves, are really immense societies composed of innumerable minute units which are the individual living cells. We have, in this society, a governing authority, the central nervous system; a postal-telegraphic system, the peripheral nervous system; a laboring class, the muscles and glandular tissue-cells; distributing agencies, the blood and lymph, and with all of these not a rigid central autocratic control, but a very considerable degree of local autonomy. Every cell is working, not by deliberate instruction, but as a part of its very specialized life. In order to avoid confusion in so vast a complex of semi-independent units, numerous coöperative mechanisms must be present to adjust supply to demand and effort to need. These mechanisms imply a certain correlation of distant parts; for instance, between the neuromuscular system which controls the respiratory movements, and the need of the tissues for oxygen. This correlation of different and often widely separated activities is brought about by the interaction of two distinct types of agency, nervous agencies and chemical agencies. In so far as this correlation of activities is brought about by chemical means, it will fall under consideration in this third phase of our subject.

Part IV.—In this part we will endeavor to attack the very kernel of our problem, that part of our studies which is destined to provide the ultimate foundation of the practice of medicine and in no small measure of agriculture also. The chemical phenomena which underlie, accompany or even actually constitute the living *activities* of cells will here be our preoccupation and we will incidentally study, so far as our fragmentary knowledge at this time permits, the changes which the foodstuffs or constituents of protoplasm undergo at the instant of their utilization for the furtherance of vital functions. Here we will find our most alluring problems and our least extensive knowledge, here is the region in which must occur the greatest conquests which lie before us and those which will exercise the most fundamental and far-reaching effect upon our own lives and the lives of those who will follow after us.

Part V.—In this part we will take up the study of the waste-products which ultimately result from the activities of our tissues; the ashes, the products of combustion and the debris which result from the daily maintenance and furtherance of life.

Part VI.—In this part, regarding the entire body as a chemical machine, somewhat crudely comparable to a steam-engine, we will discuss the question of the *efficiency* of the machine and the relationship of the horse-power it can develop to the nature and value of the fuel with which it is provided. It is in this connection that we will discuss data which may enable us in some measure to answer the question, what investment of particular types and mixtures of fuel will

return the greatest interest in the form of efficient work on the part of this very complex machine, a human being? (Given, of course the incalculable psychological asset of good-will.) This is the type of problem with which the allied governments and Germany were recently grappling and in proportion as we can contribute to its answer we are assisting not merely to guide civilization and humanity safely through the most dangerous crisis of all its long history, but also to solve a perennial problem which the War merely rendered acute a little earlier than would otherwise have been the case, the problem, namely, of correlating production and distribution with the fluctuating needs of the scattered populations of the world. The specialization of the occupations of peoples and areas which has so characterized the development of civilization in the past century carries with it inherent dangers which approach more and more near as the process of specialization extends. The specialized individual is always dependent upon others for his support. The specialized city or nation is dependent upon the world. The mutual dependency of peoples which our multifarious modern activities has evoked compels attention, in widely separated parts of the world, to the needs of remote and alien workers. These needs are chemical in their basis and biochemistry alone can supply us with the exact knowledge which is necessary to adjust them.

PART I.

THE FOODS.

CHAPTER I.

THE SIGNIFICANCE OF FOODSTUFFS.

THE CHEMICAL RELATIONSHIP OF ANIMALS AND PLANTS.

In considering the nature of the foods and their elaboration into living matter, it is necessary in the first place to realize that the foods of multicellular animals such as ourselves, are, at the same time, the constituents out of which living matter is built up. This becomes evident when we recollect that the majority of our foodstuffs consists of matter that was formerly living or which is derived from matter that was formerly living. Meats and vegetables and grains are, of course, matter that was awhile ago alive, that is now arrested in its function and more or less rapidly decomposing into more elementary substances, but still contains, for the most part, the components of living protoplasm. Perhaps they are not linked together in precisely the way in which they are linked together in truly *living* matter, and perhaps the fact that this matter is no longer living is attributable to this disturbance in the linkage of its constituents. Still the constituents are there, and we appropriate them, modify them in some degree, and build them up into our own tissues. Other foodstuffs, such as sugar, are directly extracted from living tissues in which they form stores or reserves of energy, for example from beets or sugar-canes, and these are likewise appropriated to our own use.

In this respect we differ very materially from the plants, the foods of which are in general very much more elementary than ours. Plants are actually able to build up living tissues out of substances which have in themselves no necessary connection with living protoplasm; out of mineral salts, water and carbon dioxide. For this reason it used to be thought that only plants possessed the power of synthesizing the actual constituents of living matter and that we, without doing any fresh construction, simply sort out and appropriate these preformed constituents and thus live in a state of parasitism upon the vegetable world.

The discovery by Schmiedeberg and Bunge in 1876 of the synthesis of hippuric acid from benzoic acid and glycocoll in the tissues of the kidney,[1] disposed of this untenable distinction, and while we are certainly to be regarded as primarily parasitic upon the vegetable and lower orders of the animal kingdom, yet we are not so unable to create constituents of living matter, as earlier investigators imagined. We know now that animal tissues can perform a multiplicity of syntheses whereby constituents of protoplasm are made which the food does not contain preformed. It will be found, however, to be a general characteristic of syntheses carried out in animal tissues, that the storage of energy or heat-value which is accomplished thereby is usually small, whereas in green plants syntheses are accomplished which involve the locking up, for longer or shorter periods, of very large quantities of energy; for eons as in coal deposits, or for the brief period of a single winter as in the seeds which consume their stored-up energy when they germinate in the spring.

The reason for this distinction is not far to seek. The green plant has an inexhaustible reservoir of energy upon which to draw; the radiant energy of the sun; and the energy which is locked up in the starches, fats and proteins, which plants synthesize from the most elementary products of combustion, is derived in the long run from the sun. The animal has no comparable capital to draw upon, and if an animal is to perform a synthesis involving absorption of heat or energy, it can only do so at the expense of its current account, that is to say by the degradation of its own tissues or food reserves. On the whole, therefore, and with the exceptions noted, green plants are the prime conservers of energy, while the function of animals is to dissipate it again. The whole fever and bustle of life upon the earth is therefore none other than a transitory phase through which continually passes a minute fraction of the colossal outpourings of solar energy.

THE CONSERVATION OF MATTER.

Whatever may be the relative efficiency of different types of protoplasm as storers of energy and creators of living matter, they are all alike subject to the law of the **Conservation of Matter.**[2] That is to say, although an animal or plant cell may create new chemical compounds; new permutations and combinations of the chemical elements, it cannot create new elements. All of the carbon in its tissues must have been derived, for example, from carbon from without. If an animal gives off nitrogen in the form of urea, it must either take up fresh nitrogen from without, or else its tissues must remain permanently poorer in nitrogen.

[1] That this synthesis occurs in some organ or tissue of the body had been recognized by Wöhler as early as 1824.

[2] The applicability of the law of the Conservation of Matter to living organisms was first demonstrated by its discoverer, the French chemist Lavoisier (1743–1794), and subsequently confirmed in detail by Liebig (1803–1873).

Now it is self-evident that we are continually voiding waste products, urea and very many other substances in the urine, carbon dioxide and water-vapor in the breath, and various items of waste in the sweat and in the feces. Furthermore, despite this rapid and continual loss of substance, when we are adult we remain tolerably constant in weight and composition, that is, if we are healthy and neither becoming emaciated nor growing fat. It follows that, as a general rule, we must be taking in from without, not only just as much total substance as we are losing daily in these various ways, but also just as much of each of the individual elements, nitrogen, carbon and so forth, as we are daily voiding. This intake of elements constitutes the act of feeding, and the forms in which we take in these elements are our **Foods.**

THE CLASSIFICATION OF FOODSTUFFS.

As has been stated, our articles of diet are more complex than those of the plants. Plants can utilize the carbon in carbon dioxide, but we, in order to replace our carbon-waste, must use some more complex compound of carbon, in fact, as our daily experience reveals, either a carbohydrate (sugars, starches, etc.), a fat, or a protein. Otherwise we inevitably suffer from carbon starvation. Plants, again, can derive nitrogen from nitrates in the soil, but we, more dependent, can only derive the nitrogen which we need from preformed protein. Mineral and other inorganic foods we only utilize to replace or provide mineral or inorganic constituents of our tissues; we cannot utilize them directly to build up carbohydrates or fats as plants can. Our foodstuffs fall, therefore, into four main classes, to wit:

1. The Inorganic Foods, such as water and mineral salts.
2. The Carbohydrates, such as the sugars and starches.
3. The Fats.
4. The Proteins.

To which must be added certain accessory articles of diet, to which frequent reference will be made, which are of vital importance to the maintenance and furtherance of life, but yet do not necessarily fall within any of the above-mentioned classes.

CHAPTER II.

THE INORGANIC FOODSTUFFS.

WATER AND SODIUM CHLORIDE.

We can readily understand how the need for the organic foodstuffs arises: the fats, carbohydrates and proteins. For they are fuels which in the course of combustion give up a certain number of heat-units which can be utilized in the performance of all the work which an animal daily accomplishes. We can also readily understand how the need for **Water** arises. Protoplasm consists very largely of water. Over 70 per cent. of our body-weight is water and consequently we living animals are reservoirs or sacks of water which are at the same time porous. Just as an earthenware jar containing water will gradually but continuously lose the water by evaporation from the outer surface of the jar, so we also lose water continually, by evaporation from the skin and from the respiratory epithelium in the lungs, apart from the water which is daily lost in urine and which serves the purpose of flushing the excreta out of the conduits of the body. Consequently a need for water arises, a need of the cells and tissues which is expressed in our consciousness by that indefinite sensation which we call "thirst."

But it is not so clear why we should require **Mineral Salts.** We do not decompose them. They can yield us no energy. It is not at once evident why we should lose them as we cannot help losing water. Yet we do lose them daily and that daily loss must be replaced. We daily take in sodium chloride and it reappears as sodium chloride in the urine. At the end of its passage through the tissues it appears unaltered, yet it has unquestionably performed a function and indeed many functions during its sojourn in our bodies.

The probable nature of some of these functions will be more clearly apprehended at a later stage, when we take up the consideration of the relationship between the properties of living matter and those of its constituents. But having regard at present only to the beginning and the end of the cycle of processes in which the mineral salts of the diet take part as they pass through the body, the question presents itself: what is the daily loss of mineral salts and what must be the daily intake to recoup the body for this loss?

The mineral salts which are found in our tissues are, for the most part, supplied in abundance in our diet. We do not consciously seek for them as desirable in themselves. A remarkable exception to this rule is afforded by common salt, sodium chloride, of which we feel

impelled to seek an additional supply. This fact is the more remarkable because all of our ordinary articles of food contain abundance of sodium chloride, yet however much of other diet we may eat we still experience salt-hunger, a hunger which under certain conditions, may become positively distressing.

In this connection it is noteworthy that a very close parallelism exists between the nature of the diet of different animals and peoples and their requirement of salt; a parallelism which was first pointed out and interpreted by the physiological chemist von Bunge.

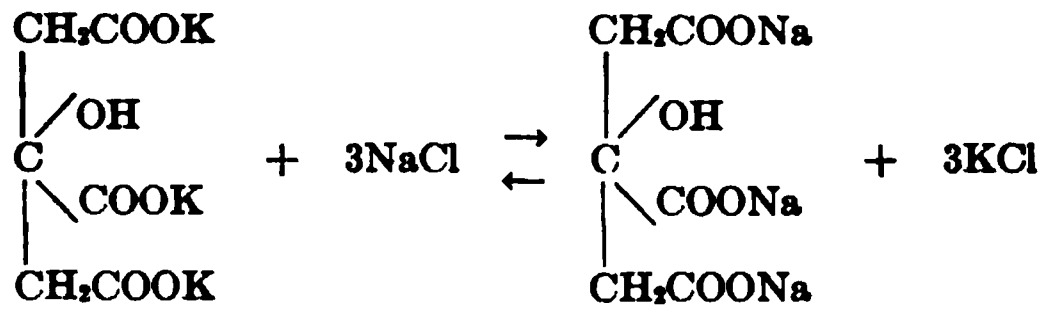
Very many of the animals whose diet is purely vegetarian experience a desire for salt. Carnivorous animals, on the contrary, such as the dog or cat, not only do not desire salt, but actually exhibit an aversion for salted food. This is very well illustrated by well-known habits of many of the wild animals. It is a fact commented upon in nearly every book of traveller's and hunter's tales, that the hoofed animals, the deer and so forth, of which the dietary is exclusively vegetable, deliberately seek for salt, in salt-pools and efflorescences, where they lick the salt, and will travel very long distances to do so. As all readers of travel and adventure know, it is at salt licks that hunters watch for such game. On the other hand, salt has never any attraction for the wild beasts of prey.

This difference of behavior becomes all the more striking when we reflect upon the fact that, weight for weight, a herbivorous animal takes in with its ordinary food just about the same quantity of sodium and chlorine per day as a carnivorous animal. Each receives the same allowance of salt. Yet the herbivora experience a longing for more salt and the carnivora do not.

The reason is obviously to be sought in some other difference between their contents of sodium chloride. Now one very striking difference is found between the mineral contents of vegetable and animal food. Vegetables nearly all contain a superabundance of potassium salts. Animal flesh, on the contrary, contains sodium and potassium in nearly equal proportions, so that although a herbivorous animal obtains just as much sodium chloride per day as a carnivorous animal, yet it obtains in many cases no less than six times as much potassium as a carnivorous animal does. It is in fact a general rule, to which there are but few exceptions, that in plant-tissues potassium predominates very greatly over sodium, while in animal tissues these mineral bases are present in approximately equal proportion.

Von Bunge sought to trace the origin of the craving which herbivorous animals experience for salt to the excess of potassium in the diet. Suppose that a salt of potassium, say potassium citrate, gains entrance into the blood by having been ingested with the food. On arriving in the blood-stream, the potassium citrate meets with an excess of sodium chloride, for in the blood-plasma, or fluid part of the blood, sodium predominates very greatly over potassium. Of course a certain degree of interchange of ions will take place. A proportion of the potassium

citrate will react with sodium chloride to form potassium chloride and sodium citrate, in accordance with the equation:



and an excess of sodium citrate appears in the blood together with an unusual excess of potassium chloride. Of course a similar interchange would take place, with analogous results, if the salt ingested were potassium tartrate, malate or any other of the organic salts of potassium which are so frequently abundant in vegetable tissues.

Now it is a function of the kidneys, as the reader will soon come to appreciate very fully, to keep the composition of the blood very nearly constant. They act, in fact, with the utmost precision, picking out and rejecting abnormal or excessive constituents. The composition of the blood cannot vary beyond the slightest extent without the supervention of grave disturbances involving all the tissues of the body. As a result of the ingestion of potassium citrate, tartrate, malate or other potassium salts which are found in vegetables, we have seen that a new salt of sodium is formed in the blood-plasma, to wit, sodium citrate, tartrate, malate or what not. This abnormal constituent is straightway picked out and eliminated by the kidneys, together with as much as possible of the excess of potassium chloride, and thus as a result of the ingestion of potassium salts the blood is robbed of both sodium and chlorine.

This theoretical deduction can very readily be illustrated experimentally. Von Bunge collected his urine from day to day and measured the diurnal excretion of sodium. He then simply added 18 grammes of K_2O, in the form of citrate or phosphate, to his daily diet. The twenty-four-hour excretion of sodium (estimated as Na_2O) immediately increased by 8 grammes. Now 18 grammes of K_2O is not at all an unusual amount to ingest along with a vegetable diet. If one were to satisfy one's protein requirements with potatoes, as many Irish peasants do, for example, one would obtain no less than 40 grammes of K_2O per diem.

One result of subsisting upon a vegetable diet, therefore, is a continual abstraction of sodium and chlorine from the blood. Now the blood resists most strongly any alteration in its composition. The reader will come to appreciate more and more clearly as this work progresses, how intimately the most fundamental activities of the body are dependent for their continuance upon the unalterable composition of the blood. The slightest alteration even in the ratio of sodium to potassium in the blood would work havoc with our tissue-activities. Hence the blood must recoup itself, and it can only recoup itself by

abstracting sodium and chlorine from the tissues. Hence the tissues, in consequence of a vegetable diet, are robbed of sodium chloride. They experience salt-hunger, a want which finds psychological expression in an indefinable longing for things which taste salt.

That the desire for salt which so many herbivorous animals experience is really attributable to the nature of their diet is remarkably illustrated by the habits of various human races. Von Bunge has collected together by exhaustive inquiries from travellers, explorers, and works of travel, a quantity of information regarding the consumption of salt among different peoples. Only to cite a few among very numerous instances: Country people, in Europe at all events where habits have become fixed by centuries of adherence to the soil, eat more vegetables and less animal food than the dwellers in cities. For instance in France, where the collection of internal revenue upon salt facilitates the acquirement of statistical data, it has been found that the consumption of salt per head is three times as great in the country districts as in the cities. Then there are whole tribes of nomads in various parts of the world who are hunters, such as certain tribes of the old North American Indians, some Arabian and Siberian tribes and the Bushmen of South Africa. These people live, or used to live exclusively upon a flesh-diet and they never taste salt. In fact, as a rule, they find salt very disagreeable and consider the use of it by Europeans ridiculous. Not only is this the case with tribes who have lived for generations upon a flesh-diet, but it applies also to Europeans who visit them and adopt their diet. Thus one traveller informed von Bunge that while he lived among the Tunguses, an exclusively carnivorous tribe which dwells in Siberia, he lived entirely upon reindeer-flesh and game, and never experienced the slightest desire for salt or inconvenience from the lack of it.

Very different was the experience of the Scotch explorer, Mungo Park, when travelling among the negro tribes of West Africa. These people live upon a mixed diet containing a very high proportion of vegetables. Salt is very rare in their country, and, as the vegetable diet causes a longing for salt, Park states that among the natives, to say that a man eats salt with his meals was equivalent to saying that he was rich. In Park's own words: "In the districts of the interior salt is the greatest of all delicacies. It strikes a European very strangely to observe a child sucking a piece of rock-salt as if it were sugar. I have frequently seen this done. I myself have found the scarcity of this natural product very trying. Constant vegetable food causes a painful longing for salt that is quite indescribable. On the coast of Sierra Leone the desire for salt is so keen among the negroes that they gave away wives, children, and everything that was dear to them, in return for it."

Hunting tribes, therefore, who subsist on flesh, experience no need for salt and never eat it even when it is easy to obtain. Agricultural tribes, on the contrary, experience a keen desire for salt. A peculiar

confirmation of von Bunge's interpretation of this phenomenon is afforded by the custom of one tribe to which von Bunge refers, the negro inhabitants of a region in the neighborhood of Khartoum. These natives manufacture or formerly manufactured a salt of their own, by igniting the ash of a plant belonging to *Salsola* or salt-wort group. As has been stated, the majority of plants contain a much larger proportion of potassium than of sodium. The plants of the Salsola group are quite peculiar in the respect that their ash contains a much higher proportion of sodium than of potassium. The employment of this particular plant-ash among all the others that might have been tried can hardly be considered accidental, in other words it must have been found to satisfy a desire not equally readily satisfied by the ashes of other plants.

The relation of a need for salt to the partaking of a vegetable diet has had several peculiar historical consequences. For example, in the Mosaic Law, the Jews are expressly commanded to present their vegetable offerings to the Deity accompanied by salt. In Greek and Roman times, sacrificial animals were offered up to the Gods without salt, but the fruits of the earth with salt.

The effect of eating salt with our food is therefore, to widen the circle of palatable foods, We all know how insipid potatoes taste without salt. That is probably attributable to their high content of potassium, unusual even in plants. By adding salt to our diet we are able to render potatoes palatable, and so with many more foodstuffs of vegetable origin.

So far, all of the facts which we have cited are in excellent harmony with the view that a diet containing an excess of potassium salts gives rise to a necessity and a desire for common salt. Not every animal appears to experience this desire, however, for rabbits and hares, for example, live on a diet containing an excess of potassium salts and yet do not seek for salt and do not appear to experience any inconvenience from lack of it. Domestic herbivorous animals will live without inconvenience on a purely vegetable diet without salt indefinitely, although they will eat salt when it is offered them and unmistakably find it gratifying. None of these live on a diet so excessively rich in potassium as potatoes, for example, but nevertheless there is no question but that they must ingest a large excess of potassium salts. Yet the blood-plasma of such animals remains of the usual composition, containing an excess of sodium over potassium.

Here we meet for the first time with a phenomenon which is of very general occurrence in living matter, namely the phenomenon of selective assimilation by tissues. Living tissues, as we shall have occasion to note many times, are not mere passive recipients of whatever may be contained in the fluids which bathe them. They choose and select suitable ingredients in suitable proportions and reject unsuitable or excessive ingredients. A remarkable illustration of this is afforded by an experiment of Landsteiner's. He fed young rabbits upon meadow

hay exclusively for three and a half months. At the same time a similar batch of animals was fed exclusively upon cow's milk. Now these two diets contained very different relative amounts of sodium and potassium, hay being much richer in potassium than in sodium, and milk richer in sodium than in potassium. Yet at the end of the period the composition of the blood obtained from the two groups, as regards sodium and potassium, was identical. The tissues, not only the epithelium of the kidney but that of the intestine as well, actively choose the constituents which they will reject or absorb respectively. In just the same way a plant, living in water rich in sodium and poor in potassium, will nevertheless pick the potassium out and build it up into tissues which are rich in potassium and poor in sodium. But this power of selection is limited, and in extreme cases, as, for example, a diet so rich in potassium as potatoes, some aid is required, and sodium and chlorine in the form of common salt must be added to the dietary.

From the standpoint of physical chemistry it is of course evident that selective absorption of mineral salts by the epithelium of the intestine or their selective elimination by the kidneys must involve the performance of work; the expenditure of energy. For the osmotic pressures of the various salts in the solutions bathing the cells would tend to drive them into the absorbing or excreting tissues in proportion to their concentration and if, on the contrary, they appear on the other side of these epithelial tissues in emphatic disproportion to their original concentrations, the process of assimilation or excretion must have involved the overcoming of the forces of **Osmotic Pressure.** The energy necessary to achieve this can only be derived from the combustion of other foodstuffs or constituents of tissues which are thus robbed of the supplies available for carrying on the other activities of the body. Selective absorption or excretion implies work, therefore, and anything which relieves the tissues in any measure of the necessity of exercising selection sets free a certain number of heat-units for other uses or, in other words, improves the utilization of other foodstuffs. The gratification and frequent improvement in nutrition which accompanies the administration of salt to herbivorous animals may thus originate in relief of the tissues from the strain and burden of selection and the liberation of foodstuffs for the maintenance of other tissue-activities which is in effect, equivalent to the addition of a certain amount of food to the accustomed dietary. The beneficial effects of salt may therefore, and in the long run, reside not so much in the actual sodium and chlorine administered as in the additional carbohydrate, fat, or protein which is thus rendered available for the maintenance and upbuilding of the body.

It is probably for some such reason as this that the total mineral-requirements of the body vary exceedingly with the dietary upon which an animal is subsisting. Especially is this the case when the requirement on a normal mixed diet is contrasted with that which obtains when the diet is limited in such a way as to provide only those proteins

of vegetable origin which are most remote in their composition from the proteins of animal tissue. In such a diet a large proportion of the nitrogen is wasted because, as we shall see in a subsequent chapter, the amino-acids into which the protein splits up on digestion are present in the wrong proportion and have to be resorted and selected in very different proportions in order to build up proteins of the animal type. It has been found that an animal subsisting on a diet of this kind suffers not only a large wastage of nitrogen, necessitating the consumption of a large quantity of food to maintain nitrogenous equilibrium, but also a large wastage of mineral constituents, so that it cannot be maintained in health or nutritive equilibrium without the addition to the diet of a considerable excess of mineral substances over the amount which would be required by an animal subsisting on a more varied diet.

CALCIUM.

During the early months of the growth of a suckling infant or animal, lime is very rapidly being absorbed and utilized by the tissues for the formation of bones. This calcium is totally derived from milk. Now the lime in milk is present therein in two forms, namely, in the form of calcium phosphate and in the form of a bulky, indiffusible compound with one of the proteins of milk, casein. The calcium phosphate is, of course ionized, but the calcium caseinate, on the contrary, does not yield calcium ions in solution.

When we add acids to milk, or when owing to the action of bacteria upon the milk-sugar which it contains lactic acid is produced in the milk, it assumes the curdled appearance which we are accustomed to associate with "sour milk." This appearance is due to the separation of free **Casein**, uncombined with calcium, which has been abstracted from the calcium caseinate by the acid. Free casein is insoluble in water or very dilute acids and hence is precipitated in curds or flocculi, while the calcium is now present in the "sour" milk in the form of the calcuim salt of the acid which has been added.

Precisely the same thing happens when milk which has been ingested by the suckling comes into contact with the hydrochloric acid which is contained in the gastric juice. Free casein, more or less modified by partial digestion, is precipitated and calcium is set free as calcium chloride.

There has been a good deal of discussion in the past as to whether the two forms of lime in milk are equally readily utilized by the suckling. In view of the above-mentioned facts there would appear to be no very good reason for distinguishing between them, since in the stomach, where absorption begins, both forms of calcium are reduced to a common level by the conversion of the calcium caseinate into the ionized and diffusible chloride.

Notwithstanding this fact it has been frequently argued that the

calcium which is combined with casein in milk is of superior nutritive value to that which is present in the milk from the beginning in the form of diffusible inorganic salts of lime. An experiment which used to be frequently quoted in support of this view was that of Lunin's; who fed six mice upon a mixture of casein, fat and cane-sugar plus the inorganic salts contained in milk. These animals lived respectively twenty, twenty-three, twenty-nine, thirty and thirty-one days; whereas two mice of the same age fed entirely upon whole cow's milk for a period of seventy-five days remained in good health at the end of the experiment. In the first experiment the inorganic bases were all combined with inorganic acids to form diffusible and ionizable salts, whereas in the second experiment the lime, at least, was combined with casein. Hence, it was argued, lime in the inorganic form did not fulfil the necessary requirements of the animals.

This experiment might easily have been seen from the first to be inconclusive, for natural milk and an artificial mixture such as that prepared by Lunin must obviously differ in many particulars besides the single particular of the diffusibility of the calcium. But in the light of our more recent accessions of knowledge concerning the nutrition of animals it has become quite clear that Lunin's experiment bears a very different interpretation to that which was originally put upon it.

We know now, thanks to researches which will be detailed in a later part of the work, that besides a sufficiency of proteins, fat and carbohydrates, any diet which is to maintain animals in health for a considerable period must contain other essential constituents which are present in milk or in animal tissues in minute amounts. These constituents fall into two distinct classes, at least, and possibly as our knowledge increases will be found to be more numerous and more diverse in their chemical characteristics. than we at present realize. The two classes of these "accessory foodstuffs" which are at present recognized, however, are in the first place the vitamines, which are nitrogenous, water-soluble substances and in the second place a group of substances which are commonly found associated with animal fats, but are generally absent from vegetable fats. Thus Hopkins has found that if animals be fed for a considerable period on milk-salts, casein and milk-sugar they will not survive, while the addition of a small amount of butter suffices to render the diet adequate for the needs of the animals.

In the light of these facts it will readily be seen that Lunin's experiment does not bear on the question of calcium-nutrition at all, but rather on the question of accessory organic foodstuffs. Furthermore, recent experiments have shown that the cane-sugar employed by Lunin in his artificial mixture is not by any means a sufficient substitute for milk-sugar in the dietary of young animals.

There is thus no evidence whatever that the two forms of lime in milk are not equally available and useful to the suckling, as we should

expect them to be from the fact that they are alike diffusible and ionizable very shortly after they arrive within the stomach.

These considerations have an important bearing upon the practical puestion of the modification of cow's milk for infant-feeding. It is the common practice to add lime-water (calcium hydroxide solution) to milk for young infants for two purposes; in the first place in order to delay the acidification and consequent "curdling" of the milk by the hydrochloric acid in the stomach. This results in deferring the flocculation of the casein until it has undergone partial digestion by the rennin and pepsin in the gastric juice, when the flocculi which are formed are finer and more gelatinous and therefore more easily penetrable by digestive juices than they are if curdling occurs without preliminary digestion. In the second place the lime-water is added with a view to increasing the supply of lime to the infant and thus assisting the growth of bony tissues, teeth, etc. From the latter point of view this practice has been decried in some quarters, on the ground that lime which is not organically combined is not so readily assimilated and utilized as calcium which is in organic combination. We have seen that there is no experimental justification for this distinction, and even if there were, the lime-water which is added to milk immediately combines in considerable proportion with the casein to form a compound of exactly the same type, only richer in calcium, as that which is found in normal milk, so that the greater part of the calcium thus administered does in fact reach the stomach in a state of organic combination.

It is, of course, quite another question whether administration of lime beyond a certain daily amount is of any value in assisting the growth of bony tissues. Experience in connection with other articles of diet conclusively shows us that in many instances the effective administration of foodstuffs is limited by the ability of the tissues to utilize and elaborate them, any supply in excess of this being rejected and wasted. Defective development of bony tissues may be sometimes attributable to deficiency of lime in the diet, but it is probably more often due to inability of the bone-producing tissues to utilize the lime which is presented to them. This, however, obviously constitutes no objection to the addition of lime-water to the milk of an infant; it merely indicates a reason why this procedure by itself may often be insufficient to correct faulty or deferred development of the calcareous tissues.

The lime-requirement of the adult is very greatly increased in the female by activity of the mammary glands. Thus from 0.3 to 0.5 gramme of calcium oxide per hundred pounds of body-weight per day is sufficient to supply the minimum needs of a pig or goat which is not yielding milk, but a milch-goat requires an additional 1 to 2 grammes of calcium oxide per day for every pound of milk it yields. Insufficiency of lime in the diet under such circumstances results in actual withdrawal of lime from the skeleton, a condition which when it becomes sufficiently

acute to cause softening and bending of the bones is known as **Osteomalacia.** It is not to be inferred, however, that osteomalacia is always due to deficiency of calcium in the diet. It may be due as indicated above to physiological disturbances or nutritional deficiencies leading to faulty utilization of the calcium which the dietary affords.

Calcium is excreted, in part by the kidneys and in part by the intestinal mucosa. A high proportion of soluble phosphates in the diet tends to increase the output of calcium in the feces, probably owing to the formation of calcium phosphate which is insoluble in the alkaline fluids of the intestine. Just as potassium salts increase the output of sodium in the urine, so, and for similar reasons, do magnesium salts increase the output of calcium in the urine.

IRON.

Iron is an essential constituent of the red pigment of the blood, **Hemoglobin.** Since hemoglobin is the carrier of oxygen from the lungs to the tissue-cells, it is obvious that iron in this, if in no other capacity, plays a vital part in the economy of the body, but, in addition to the hemoglobin-iron, iron is also found, and not necessarily associated with hemoglobin, in other parts of the body. Thus the liver contains about 0.02 per cent. of iron calculated on the basis of the fresh, undried organ washed free from blood. The muscles contain appreciable quantities of iron, especially heart-muscle, which contains about 0.01 per cent. of the fresh, undried weight. In smaller quantities iron is found elsewhere in the body, regularly accompanying **Nucleins** and **Nucleoproteins** wherever they are found.

The iron-content of the adult is subject, like that of other tissue-constituents, to daily losses. Experiments with starving individuals (and it is under conditions of starvation that the body is most economical of its resources) show that the nominal daily loss of iron in the feces is from seven to eight milligrammes, while in addition to this a daily loss of about one milligramme occurs through the kidneys. In all, then, it is probable that about ten milligrammes of iron, or about one three-hundredth of the total hemoglobin-iron in the body is lost per day. This loss must be replaced from the diet.

Under certain pathological conditions, or conditions of malnutrition, a loss of hemoglobin occurs from the blood and the patient is said to have become "anemic." This loss of hemoglobin may and on the other hand may not be accompanied by a diminution in the number of red blood-corpuscles. As might be anticipated, the result of this condition is suboxidation in the tissues with consequent symptoms which are sometimes of the severest gravity. These are very well illustrated by the chlorosis, or "green sickness" which very frequently overtakes girls at the age of puberty. From periods of remote antiquity antedating by many centuries our knowledge of the chemical composition and significance of hemoglobin, this disease has been combated

by the administration of inorganic salts of iron, and often with beneficial effect. For long it was thought, without any question, that the salts of iron so administered were absorbed and that the beneficial effect of the medicament was due to the replacement of the iron in the blood by the iron so administered. Doubt was thrown upon this explanation by the discovery that iron is eliminated from the body in the feces. Doses of inorganic salts of iron, administered to healthy individuals, were recovered apparently unaltered in the feces, and from this fact the erroneous conclusion was drawn that inorganic salts of iron are not absorbed. The beneficial effects of iron in anemia were either denied, a denial in which practising physicians declined to share, or else accounted for by the irritant action of the salts of iron upon the epithelium of the intestinal tract. A mild irritation has a well-known "tonic" effect which is rather difficult to define in precise terms; but which is frequently manifested, not only by increased activity of the tissues which are stimulated, but also of other and sometimes distant tissue. The beneficial effects of iron were therefore attributed to increased activity of the tissues resulting in increased assimilation and utilization of the organically combined iron in the diet and not to direct assimilation of the iron administered as a medicament.

Much has been done to clear up this question by the employment of microchemical tests to trace the course of iron through the intestine. When mice are fed upon milk alone for a considerable period, on placing the alimentary canal of these animals in ammonia and ammonium sulphide the characteristic precipitate of iron sulphide does not appear, or at the most there is only a very slight green coloration. Now milk is one of the articles of diet which is poorest in iron, cow's milk containing only about 2.3 mg. of iron per 100 grammes of dry substance. Very different results are obtained if the mice are fed upon milk to which inorganic salts of iron have been added. In the stomach there is little if any reaction for iron, while in the duodenum there is a marked green coloration. If the tissues of the intestine are examined under the microscope, little granules of iron are found imbedded in the protoplasm of the intestinal epithelium, and leukocytes are found laden with minute particles of iron. In the jejunum, however, and in the ileum, very little iron is found, while in the cecum and large intestine a strong iron-test is once more obtained.

Coming from the intestinal canal, especially from the duodenum, the lymphatics may be seen filled with cells containing iron. The liver and spleen give much stronger tests for iron than those of the mice fed upon milk alone.

There can be no question, therefore, but that the inorganic iron-salts thus administered are absorbed. Part of the iron appears to be conducted by way of the lymphatics to the thoracic duct and the blood-stream. Part is unquestionably conducted by the portal vein to the liver, which is a storehouse of iron as it is of many other things. The absorption takes place mainly in the duodenum; the excretion of waste

iron occurring, on the contrary, in the cecum and large intestine, although part of the small intestine may also participate in this function.

It is one thing to show that inorganic salts of iron are absorbed and it is another to show that they may be utilized in the building up of hemoglobin. The iron in hemoglobin is very firmly and intimately combined, and cannot be detected by the reagents ordinarily employed for this purpose, such as ammonium sulphide or potassium ferrocyanide. In fact the iron in hemoglobin resists the action of boiling, concentrated potassium hydroxide and boiling hydrochloric acid. Only by dissolving the hematin radical (which is the iron-containing moiety of the hemoglobin molecule) in concentrated sulphuric acid is the iron split off and the hematin changed into iron-free hematin, or **Hematoporphyrin.**

Most of the iron in our diet is in the form of hemoglobin or other organic compounds of iron from which free ionic iron is not readily split off. The yolk of eggs is very rich in iron, as might be anticipated from the fact that the yolk of an egg must contain all of the constituents necessary to form the hemoglobin of the developing embryo. The iron-compound in yolks of eggs is not hemoglobin, but some antecedent of hemoglobin. On extracting the yolk of a hen's egg with alcohol or ether, none of the iron goes into the extract. The residue, which contains all of the iron, is a mixture of proteins and nucleoproteins. The iron cannot be extracted from this residue by alcohol and hydrochloric acid, although inorganic salts of iron readily yield up iron to these reagents. During the digestion of iron-containing protein by **Pepsin** in the stomach, the part containing iron does not go into solution and its digestion is not accomplished until it reaches the small intestine and comes in contact with the digestive fluid secreted by the pancreas. It is not digestible by pepsin and in this and in other respects corresponds in its behavior to the class of bodies which the reader will later learn to recognize as nucleins. The ordinary tests for iron are given by this substance, to which von Bunge gave the name "**Hematogen**," but not so readily as by inorganic salts of iron. On adding ammonium sulphide to an ammoniacal solution of this nuclein a greenish coloration is produced, which only slowly changes to black on standing. In other words ionized iron is at first only present in traces and is slowly split off from the compound under the prolonged influence of the reagents. The compound thus behaves in a manner very like that of the protein salts of the heavy metals, for instance casein salts of silver, mercury and so forth to which the reader's attention will be directed in a later chapter. There is little reason to doubt that hematogen is simply a protein salt of iron in which the protein is acting the part of a weak acid, or else a double salt of protein and an inorganic salt of iron. Protein compounds of this type yield no metal-ions in solution, or at the most, only traces of them.

Since compounds such as these are the only forms in which we

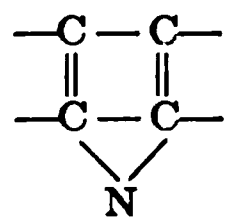
normally receive iron in our diet, for we only partake of inorganic salts of iron as a therapeutic measure, there can be no question but that we can absorb, assimilate and utilize the iron contained in organic, non-ionized compounds.

It will be recollected that the iron in hemoglobin or hematin does not yield the ammonium-sulphide test for iron. On administering hematin or hemoglobin to mice which have undergone iron-starvation, however, and applying the iron-sulphide test to various parts of the intestine, we ascertain the remarkable fact that the duodenum and the cecum yield the iron-test just as they do when inorganic iron is administered. In other words, the iron in the process of digestion in the duodenum has become loosened from its combination in the hematin radical and set free as an inorganic or at least an ionized salt of iron. Since the iron, immediately subsequent to absorption, appears in the same condition whether administered in the ionic form or not, there would appear to be no very good reason for supposing that inorganic salts of iron are not utilized to nearly as great an extent as the organic salts of iron. The most specific disadvantage which attends the use of inorganic salts of iron is their irritating or corrosive action upon the intestinal epithelium, a corrosive action which, like that of mercury salts, is probably to be attributed to the formation of insoluble protein salts of the metal within the epithelial cells. This leads to the disruption of the gelatinous structures of the cells and their conversion into granules or flocculi which, no longer being held together by the cohesiveness of a jelly, fall apart with consequent disintegration of the cells. Many individuals who display an "idiosyncrasy" or exceptional sensitiveness to intestinal irritation are very severely affected by this corrosive action of iron-salts and for this reason the general employment of non-ionized organic compounds of iron in therapeutics, such as hemoglobin or hematogen, is much to be preferred.

With the exception of the disadvantages arising from the corrosive action of inorganic salts of iron, therefore, the ionized and unionized compounds would appear, so far as the above-cited evidence goes, to be equally useful sources of iron in the diet. There are certain important facts, however, which would appear at first sight to bear out the contention that inorganic salts of iron, notwithstanding their absorption, are not utilizable for the synthesis of hemoglobin. We have seen that milk contains a very low percentage of iron in comparison with other foods, especially in comparison with green vegetables, certain fruits such as apples, and flesh. If sucklings are kept beyond the normal period of lactation exclusively upon a milk diet, they become anemic from lack of iron. If we compare rabbits which have been allowed to change to a diet of green vegetables after the normal period of lactation, with those which have been brought up upon an exclusive milk-diet, we find that the former contain much more hemoglobin than the latter. But the remarkable fact is that if we add inorganic salts of iron to the milk-diet the total hemoglobin in the animals is not

increased, although they grow much more rapidly than the similarly fed animals which do not receive iron. This would appear to indicate that inorganic salts of iron are utilizable for certain purposes in the body connected with the growth of the animals, but not for the building up of hemoglobin. This conclusion, however, would be premature.

Recent acquisitions to our knowledge of the structure of the hematin moiety of the hemoglobin molecule have shown that it contains a particular molecular grouping, namely, the **Pyrrole Group**:



which there is every reason for supposing cannot be synthesized by animals but must be obtained by them preformed, that is to say from the tissues of plants or from the tissues of animals which acquired it from plants. This pyrrole grouping is contained in small amounts in the majority of proteins and it forms a very important component of **Chlorophyll**, the green coloring-matter of plants which, as we shall see, is very closely related, chemically, to hemoglobin. It is not improbable, therefore, that inorganic iron-salts added to an exclusive milk-diet are not utilized for building up hemoglobin simply for the reason that other component parts of the hemoglobin molecule, as essential as iron itself, are either lacking altogether in the milk-diet or present therein in insufficient amount to subserve the needs of the blood-forming tissues and those of the other tissues of the body as well. We will return to this question in later chapters in connection with the chemistry of hemoglobin, and again in connection with the general problems of growth and nutrition.

The percentages of iron which are contained in several common articles of food are enumerated in the following table:

IRON–CONTENT OF FOODS IN PER CENT. OF EDIBLE PORTION, AFTER SHERMAN.[1]

Food.	Iron (Fe).	Food.	Iron (Fe).
Egg-white	0.0001	Potatoes	0.0013
Butter	0.0002	Cheese	0.0013
Whole milk	0.00024	Dates	0.0030
Apples	0.0003	Eggs	0.0030
Carrots	0.0006	Meat	0.0023–0.0033
Lettuce	0.0007	Spinach	0.0036
Cornmeal	0.0009	Oatmeal	0.0038
White bread	0.0009	Barley	0.0041
Asparagus	0.0010	Egg-yolk	0.0086
Cabbage	0.0011	Blood	0.0526
Fish	0.0008–0.0013		

It will be noted that the iron-content of spinach is very high. Spinach is also very rich in chlorophyll, as its deep green color indicates, and thus contains a large proportion of another essential constituent

[1] Chemistry of Food and Nutrition, New York, 1918.

of hemoglobin, the pyrrole radical. Chlorophyll, it is true, is indigestible by the digestive juices, but it is split up by the bacteria which inhabit the intestine, and in this way a portion of the pyrrole which it contains may possibly be rendered available for assimilation from the intestine and utilization by the tissues.

It will be recollected that if iron is administered to young animals which are undergoing iron-starvation by being kept upon an exclusive milk-diet, their growth is markedly accelerated despite the fact that the iron is not utilized to build up hemoglobin. This effect is of significance, inasmuch as it indicates that iron subserves other important functions in the body besides that of entering into the composition of the oxygen-carrying pigment of the blood. We are reminded of the prevalence of iron in nuclear elements, and led to suspect that iron plays some essential part in the functions of the nuclei. It is a noteworthy fact, however, that if iron be added, in similar amounts to those employed in the above-cited experiments, to an abundant and mixed diet, containing a normal sufficiency of iron, this acceleration of growth is not observed. Evidently beyond a certain diurnal allowance the tissues of the growing animal are not able to utilize iron for the purposes which result in the acceleration of growth. Here we meet again with a phenomenon to which reference was made in connection with the utilization of calcium. The ability of the tissues to profitably utilize the materials brought to them sets a definite limit to the amount of a foodstuff which it is of any avail to consume. It is doubtless for this reason that iron, whether in the organic or the inorganic form, is without effect in accelerating the rebuilding of hemoglobin after hemorrhage. The blood-forming tissues are able to manufacture so much hemoglobin per diem and the supply of more raw materials than they can "work up" in a day is useless.

The ultimate reason for this phenomenon, which is of such general occurrence in life-phenomena, resides undoubtedly in the multifarious variety of the chemical processes which underlie and accompany vital activities. In every detail of change which accompanies the performance of any function by living tissues not merely one chemical reaction is involved but a whole series of interwoven reactions following and depending upon one another. Now in any series of chemical changes of which the second utilizes some product of the first, the third some product of the second, and so forth, it is always the specifically slowest reaction which "sets the pace" for those which succeed it. No matter how quickly raw materials may be supplied, this "*master-reaction*" can proceed only at a certain maximum speed and succeeding reactions must wait for its products before they can seize and elaborate them. Provided then, that any article of diet be supplied in sufficiency to maintain at top speed the "master-reaction" of the series of processes into which it enters, excess of this particular item in the dietary is mere wastage and casts an unnecessary strain upon the organs of elimination.

Insufficient hemoglobin content of the blood, therefore, and any other type of maldevelopment and malnutrition may originate in either of two ways, namely, through inadequacy of the diet, or through imperfect utilization of substances which are present in abundance in the dietary. Certain mild types of anemia, probably belong to the former category and the consensus of opinion of the physicians is that these are favorably affected by administration of iron. In other types of anemia, in which the utilization of iron is defective or in which, as in the anemia of hemorrhage, the lack of hemoglobin is due to loss or destruction after it has been manufactured, we cannot expect therapeutic administration of iron to be followed by equally favorable results.

OTHER INORGANIC FOODSTUFFS.

The remaining inorganic constituents of the body will be but briefly considered at this point, some of them falling under review in other connections in later chapters. While the majority of them probably play important or even essential parts in our bodily economy, we have as yet only succeeded in a few instances in obtaining a clue to the nature of these functions.

Among the metals other than those which we have considered, **Magnesium** is, from a quantitative point of view, the most important. Magnesium is found in small quantities in all animal and plant cells, and in milk. There appears to be a rather definite relationship or proportionality between the magnesium and the calcium contents of the tissues, and from the fact that a trifling excess of magnesium, when introduced into the circulation, causes profound disturbances such as glycosuria, we may conclude that magnesium has powerful physiological actions and that in consequence even the amounts which normally occur in tissues are not devoid of physiological significance.

It is stated that traces of **Lithium** are normally found in animal tissues, and it is a much-discussed question whether or not a minute trace of **Arsenic** is a normal constituent of human tissues, the gravity of the discussion being attributable, of course, to the medicolegal significance of the question. The consensus of opinion appears to be, however, that arsenic is found in human tissues only after the administration of drugs containing arsenic or in districts where arsenic occurs in considerable amounts in the soil and water.

Among non-metallic inorganic constituents of the body, **Chlorine** plays a leading part, in the alkali chlorides of the blood and tissues and in the hydrochloric acid in the gastric juice. It is derived from chlorides in the food.

Fluorine occurs in small amounts in milk (0.00003 per cent.) and is a normal constituent of bones and teeth; it is unquestionably not devoid of significance in the formation of these tissues.

Silicon is a constant constituent of hair and feathers, no less than 40 per cent. of the ash of hair consisting of SiO_2. This is doubtless derived from silicates in the vegetable portion of our diet, silicon playing an important part in communicating rigidity to many plant-tissues. According to Drechsel the silicon in feathers exists therein in a state of organic combination, as the silicate of a hydro-aromatic alcohol closely related to cholesterol.

Phosphorus is, of course, an element of prime importance in the life-economy, in the form of the phosphoric acid radical in phospho-proteins such as casein and in the form of complex substituted phosphoric acids, as **Nucleic Acid** and the glycero-phosphoric acid radical of the phosphorus-containing fats or phospholipins. This phosphorus is derived from the phosphates, phosphoproteins, nucleins and phospholipins in the diet. There is some room for question whether animal tissues utilize the inorganic phosphates in the diet for the building up of the nucleins and phospholipins. A fact which seems to indicate that animals do not depend upon inorganic phosphates for the production of these substances is that mice will grow normally and reproduce on a diet containing a high proportion of aluminum hydrate, although this results in the formation of the insoluble aluminum phosphate from any inorganic phosphates which may be present in the alimentary canal, and its elimination, without absorption in the feces.

Sulphur also plays an exceedingly important rôle, but in the form of the complex amino-acid cystine, which is a decomposition-product of many proteins, rather than in the form of free sulphates or sulphides.

Iodine is a normal constituent of the **Thyroid** and plays an essential part in the important functions of this gland. We will consider the nature of the organic combination in which it occurs and its significance in the bodily economy in a later chapter. It has been repeatedly stated that iodine is found in other tissues of the body, notably in the pituitary gland, but more recent analyses have shown that in the absence of iodide-medication, iodine is not found in normal animal tissues other than the thyroid. Iodine is an important constituent of seaweed, from the ash of which a quantity of the iodine of commerce is derived. The relatively high concentration of iodine in the tissues of these marine plants is of especial interest because the iodine content of sea-water is exceedingly low. This constitutes therefore an interesting case of the **Selective Absorption** by living tissues to which reference was made in connection with the proportion of sodium to potassium in the blood and tissues of animals.

THE COMPLEXITY OF OUR DIETARY REQUIREMENTS.

It is to be hoped that the recital of the above category of the inorganic constituents of our body, present, several of them, in the most inconsiderable traces, will have the effect of making the reader pause ere he

embraces any of the dietary fads and "systems" which are so prevalent in this uninformed and loquacious period of our social evolution. The average man or woman hesitates to pronounce an opinion on the motive machinery of steamships or aëroplanes or on the fuel-requirements of a Diesel engine, but regarding that infinitely more complex engine, a human being, the average individual deems himself fully informed and all that is required to make numerous converts to any dietetic fad is a considerable degree of self-assurance.

So complex are the requirements of the animal economy; so little do we know the parts that these several requirements play and their delicate adjustments to one another, that we are totally unable at this stage of our knowledge to enumerate the constituents of any restricted dietary which shall certainly and for prolonged periods of time, convey to the subject all that he requires for the orderly functioning of his body. In medical practice it is, of course, necessary to occasionally prescribe a limited and specified diet for a definite period in order to combat certain conditions or maladies, but to do so for lengthy periods of time, especially for growing infants and children, is to simply assume a knowledge which we do not possess. The problem of the dietary requirements, as we have seen, is complex enough when we consider only the inorganic foodstuffs; but when we add to these the organic requirements of the body the complexity of the problem of nutrition is multiplied a hundredfold, and we are as yet hopelessly in the dark respecting the source and function of a multitude of constituents of the body and of the degree to which they may be essential. Our knowledge in this field is rapidly extending, perhaps more rapidly at present than in any other field of biochemistry, but even at the present rate of accession of knowledge, the complete knowledge essential for enumeration in detail of all the dietary requisites of a human being is very far distant indeed.

The knowledge that we do possess, however, enables us in certain particular instances, as, for example, in the Weir Mitchell treatment of certain nervous disorders, or the Allen treatment of diabetes, to accomplish very decisive therapeutic results by restricted dietaries prescribed for limited periods, in conjunction with hygienic measures and adequate biochemical and clinical observation and control. The very success of such measures in any particular instance carries with it the danger of converting an ignorant patient into a fanatical diet-faddist who, upon recovery of health, proceeds to convert, first his acquaintances and then, if he has the opportunity, a wider public, to the health doctrine which he has evolved out of the temporary measures of the physician. This is no doubt the origin of many of the dietary and hygienic eccentricities to which certain genuine or imaginary invalids devote themselves. No small part of this perverted activity could probably be stifled at its birth, if the physician who is prescribing dietary or hygienic measures were to make a practise of explaining as thoroughly and simply as he is able, to the patient and his immediate

associates the precise object of the measures advocated, their temporary character, and the fact that they are applicable only to the particular case in point, and not to humanity in general, irrespective of age, sex, health or disease.

We will take up the question of the dietary requirements of the body in several subsequent chapters and in a variety of connections. The above remarks will, however, be found to apply only the more forcibly with the expansion of our acquaintance with the complexity and variety of the problems of nutrition.

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CHAPTER III.

THE CARBOHYDRATES; THE MONOSACCHARIDES.

GENERAL CHARACTERISTICS.

The **Carbohydrates** are extremely abundant in nature, and play an exceedingly important part in the life-cycle. In vegetable tissues they are of importance, not only as foodstuffs and reserve materials, but also as structural materials. For example, the walls of plant-cells are usually composed of cellulose, a complex carbohydrate. In the animal economy the carbohydrates are chiefly of importance as food and reserve-materials and afford a very important source of kinetic energy to our tissues.

The carbohydrates owe their name to the fact that all of them contain carbon and in all of them, moreover, the proportion of hydrogen to oxygen is the same as it is in water, namely, 2 to 1. This is not a very satisfactory definition of the group, however, since many substances are known which correspond to such a definition and yet are most distinctly not carbohydrates. In more exact terms it may be said that carbohydrates are aldehyde and ketone derivatives of the polyatomic alcohols. The majority of the naturally occurring simple sugars contain six atoms of carbon and are termed **Hexoses**, although some contain five atoms of carbon and are termed **Pentoses**. From the simple **Monosaccharides**, more complex sugars, the **Disaccharides**, are formed by the combination of two molecules of monosaccharide with the elimination of a molecule of water. More complex carbohydrates still, the starches and dextrines, collectively termed the **Polysaccharides**, are derived from the monosaccharides by the combination of a variable number of sugar molecules, with the elimination of a corresponding number of molecules of water.

It is only within comparatively recent times that the artificial synthesis of sugar has been accomplished, but within the brief period of thirty years nearly all of the natural sugars have been synthesized, and the light which the consequent accessions to our chemical knowledge have thrown upon the function and transformations of the carbohydrates in living organisms is so great, that today we are in a position to interpret countless phenomena which were entirely obscure before these discoveries had been made.

The first sugar to be synthesized was **Glycerose**, which was prepared by Emil Fischer in 1890. This sugar, which contained, however, only three atoms of carbon (formula $(CH_2OH)_2CO$ was prepared by the gentle oxidation of the triatomic alcohol, glycerol $(C_3H_5(OH)_3$. This synthesis is particularly interesting because it establishes a connection between the carbohydrates and the fats, since all of the naturally occurring fats contain a glycerol radical. From this sugar it was found

possible to prepare a sugar containing six atoms of carbon in the molecule, by the action of dilute alkali. At the same time Fischer succeeded in synthesizing a hexose (that is to say, a six carbon atom sugar) from its elements, by the polymerization of formaldehyde (HCHO), in accordance with the equation:

$$6HCHO = C_6H_{12}O_6$$

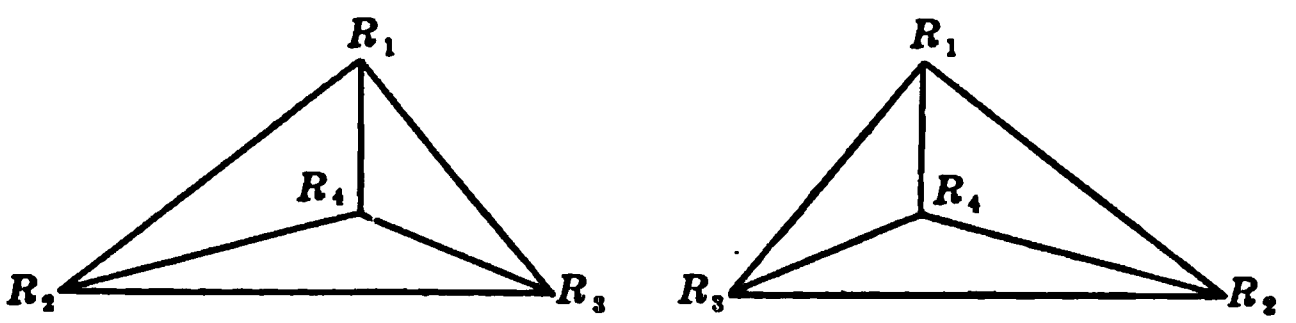
and this sugar was found to be identical with that which had been synthesized from glycerose.

Examination of this new sugar showed, however, that it differed in a very important property from the naturally occurring hexoses, fruit-sugar, glucose, or mannose. These sugars, when in solution, rotate the plane of polarization of a beam of polarized light to the right or to the left. The synthetic sugar did not rotate the plane of polarized light, and hence a special name was given to it, **Acrose.**

The reason for the optical inactivity of acrose was found to lie in the fact that it is a mixture of equal parts of optical antipodes, the one rotating the plane of polarized light to the right, and the other to the left in equal degree. As a matter of fact acrose can be decomposed by appropriate measures into optically active constituents, and according to the conditions which accompany the transformation we obtain fruit-sugar, mannose, or glucose.

It is a remarkable fact that nearly all natural products which are derived from living material are possessed in some degree of **Optical Activity.** This was at first thought to be a peculiarity of substances formed by living organisms and to point to the operation within living tissues of some force peculiar to living matter. We now understand that the optical activity of the constituents of living matter is due to the circumstance of their synthesis in the presence or through the agency of optically asymmetric catalyzers.

The exact conditions upon which this property of optical activity depends were first made clear by Le Bel and Van't Hoff in 1874. Previously to this Pasteur had expressed the opinion, based upon his fundamental observations on the differing crystal-forms of the right-handed and left-handed varieties of tartaric acid, that the optical activity of certain molecules must be attributable to a certain degree of asymmetry of the molecule. This asymmetry, in the case of carbon compounds, Van't Hoff was able to trace to the carbon atom. If we imagine the four valencies of a carbon atom to be pointing toward the four apices of a tetrahedron, of which the center is the carbon atom, the following arrangements of four different masses are possible:

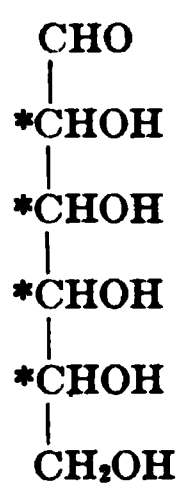


The difference between these arrangements resembles that between an image and its reflection in a mirror; the diagrams cannot be superimposed upon one another so that the corresponding parts will coincide, except by inverting one of the diagrams, and thereby converting it into the other, its mirror-image. Now it would appear that when a carbon atom is united by its valencies to four different masses, either of the above arrangements is possible, the one yielding a dextrorotatory and the other a levorotatory compound. An optically inactive body is produced either by a mixture of equal numbers of the two forms of molecules or by "internal racemization," *i. e.*, by the presence within the molecule of two equally active carbon atoms rotating the plane of polarized light in opposed directions.

This being the case, the number of possible optical isomers of a substance which contains two asymmetric carbon atoms is four, since either of the two possible varieties, levo- and dextro- of the first asymmetric atom may be combined with either of the two possible varieties of the remaining atoms. Similarly the number of possible optical isomers of a substance which contains three asymmetric carbon atoms is eight, since any of the four possible arrangements about the first two atoms may be combined with either of the two possible arrangements about the third atom, and, in general, the number of possible optical isomers of a substance which contains n asymmetric carbon atoms is 2^n.

THE HEXOSES.

The relationships which have been described above are very well illustrated among the hexoses. A large number of sugars are known which possess the formula $C_6H_{12}O_6$. The structural formulæ of these sugars have been elucidated by Fischer and others, and it has been shown that a number of these possess a structure[1] which can be represented by the general formula:



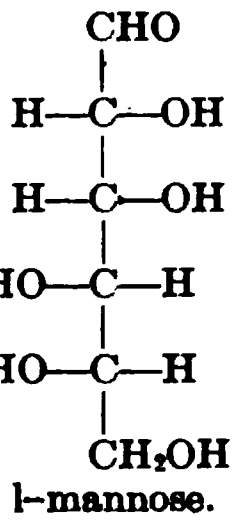
It will be observed that the four carbon atoms which are distinguished by asterisks are asymmetric, because they are each united with four different masses. For example, take the second carbon atom from the top of the diagrammatic formula. It is united with the following groups: $-CHO$, $-H$, $-OH$ and $-C_4H_5(OH)_4$. According to the rule which is enunciated above, there must be $2^4 = 16$ possible optical isomers of this compound.

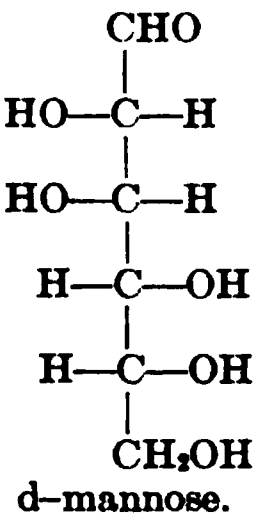
[1] Or are readily convertible into substances possessing such a structure, cf. below:

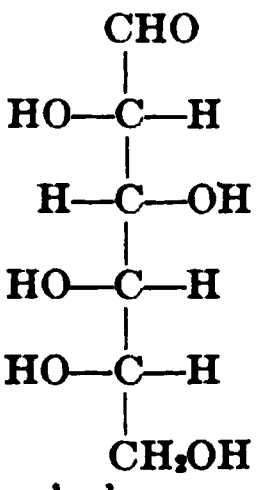
This will be rendered clearer by the accompanying diagram, which illustrates the structure of the sixteen possible stereo-isomers of any compound which contains four asymmetric carbon atoms. Designating a dextrorotatory carbon by the symbol + and a levorotatory carbon by the symbol – it will be seen that each carbon is dextrorotatory in eight isomers, and levorotatory in eight others. It is also evident that provided the end-groups attached respectively to the first and fourth asymmetric carbons are identical, the isomer number 11 is identical with the isomer number 5, 12 with 6, 13 with 7 and so forth.

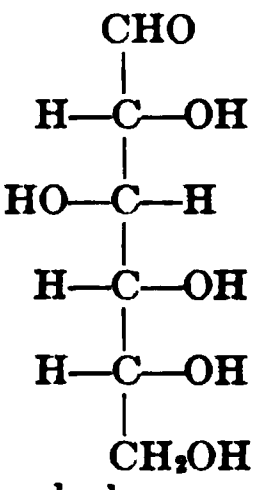
				11	12	13	14	15	16
				+	+	+	+	+	–
				+	+	+	–	–	+
				+	–	–	+	–	–
				–	+	–	–	–	–
+	+	–	–	–	+	–	–	–	–
+	–	+	–	+	–	–	+	–	–
+	–	+	–	+	+	+	–	–	+
+	+	–	–	+	+	+	+	+	–
1	2	3	4	5	6	7	8	9	10

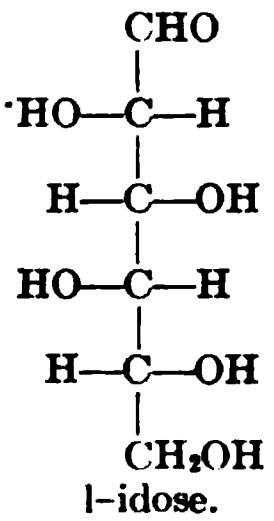
This is what actually occurs in the corresponding polyatomic alcohols, in which the –CHO group of the sugar is replaced by the group $-CH_2OH$. In the hexoses, of which glucose is a representative, the two end-groups are, of course, different and hence no two possible isomers are identical. There are, therefore, 16 possible sugars or hexoses of the aldehyde type, possessing the above formula. We may represent them as follows, using the prefixes d– and l– to signify dextro- and levo-rotatory respectively.

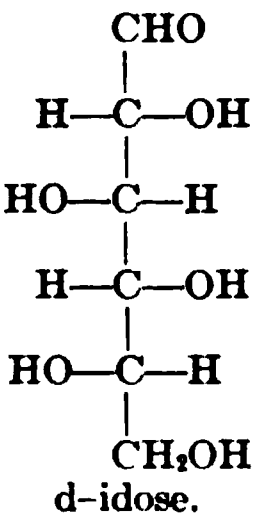

l–mannose.

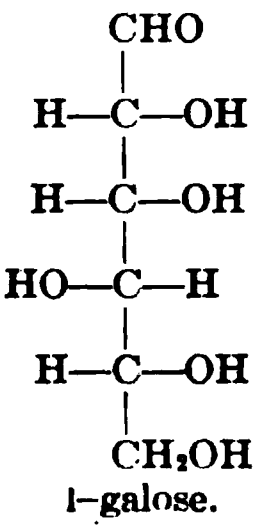

d–mannose.

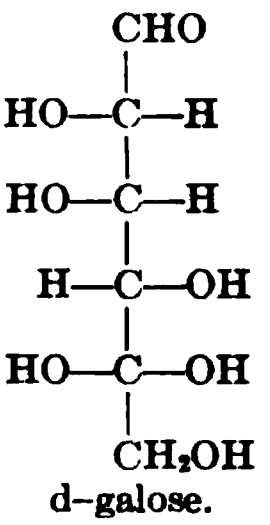

l–glucose.

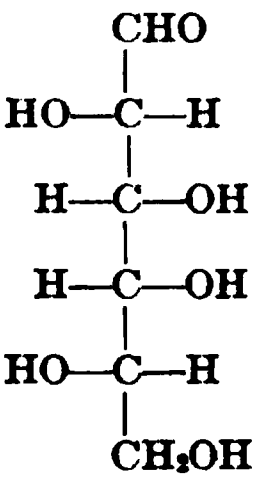

d–glucose.

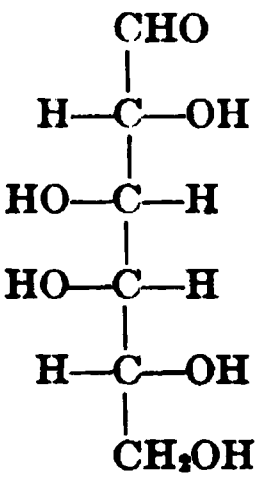

l–idose.

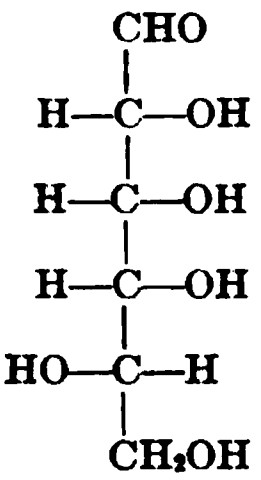

d–idose.

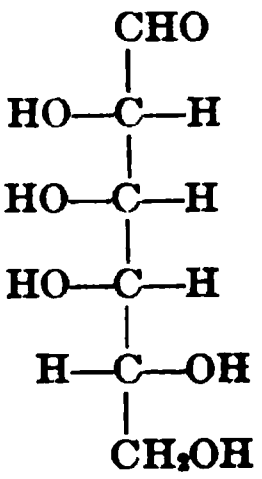

l–galose.

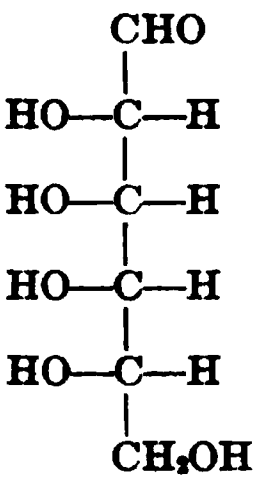

d–galose.

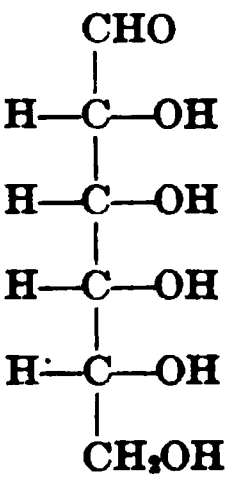

l–galactose.

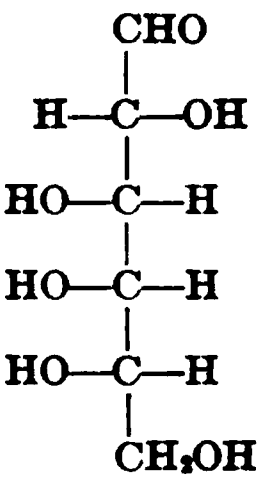

d–galactose.

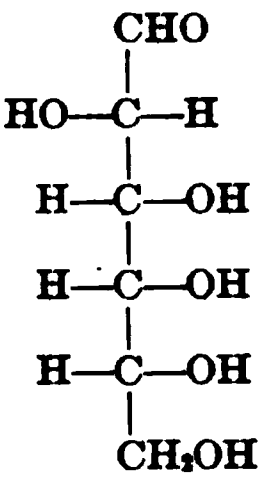

l–talose.


d–talose.


l–allose.

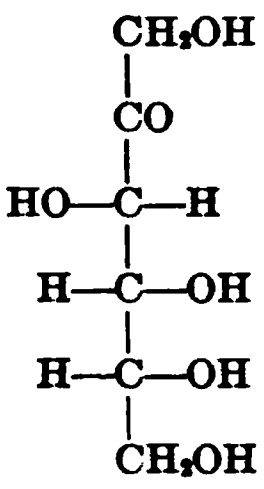

d–allose.


l–altrose.

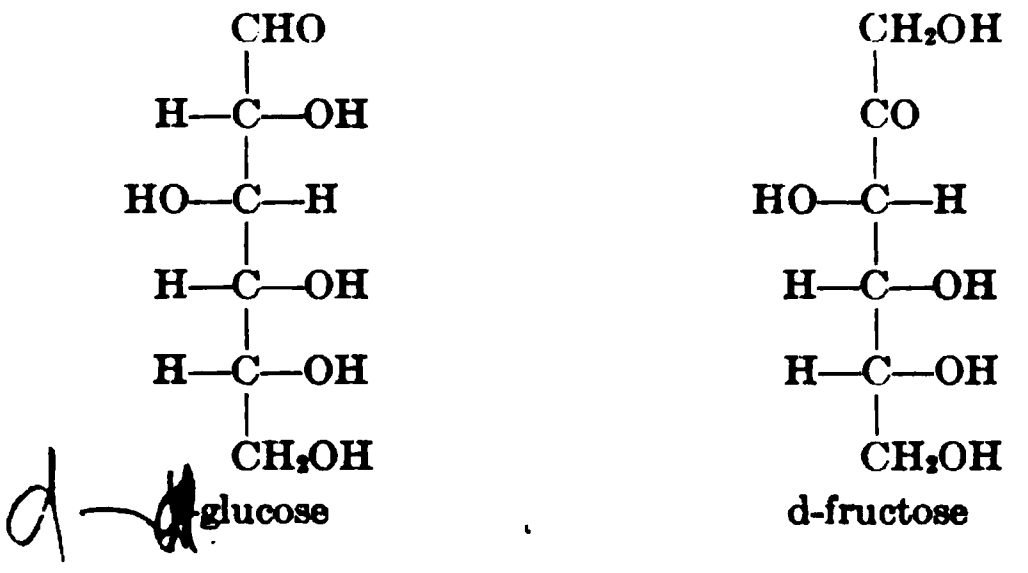

d–altrose.

The various sugars have been prepared synthetically and their constitutional formulæ confirmed. Thirteen of them are laboratory products, and only three of them are met with in nature, to wit: d– glucose, d– mannose and d– galactose.

In addition to these hexoses of the aldehyde type, or **Aldoses**, another hexose of quite a different type is of very common occurrence in nature, namely fruit-sugar or **Fructose**. Unlike all of the hexoses considered above, fructose is a sugar of the ketone type, or ketose. Its structure may be represented by the formula:

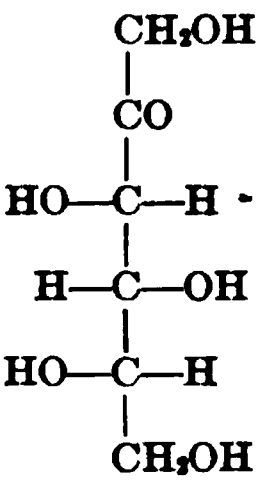


Fructose exists in a dextrorotatory and a levorotatory form, the one being the mirror-image of the other. We customarily distinguish between dextro- and levorotatory forms by the prefixes employed above, d– and l–; as, for example, d–glucose and l–glucose. The form of fructose which is represented in the formula given is the levorotary form, but it is, nevertheless, termed d-fructose, because of its close relationship to d-glucose, which will be apparent on comparing the two formulæ:



The mirror-image, which is in reality dextrorotatory, is therefore termed l-fructose. The levorotation of d-fructose has led to its being very generally designated levulose, by which name we will hereafter frequently refer to it.

Another ketose which occurs in nature is **d-Sorbinose**:



which is formed when the juice of the mountain-ash is exposed to air, by the oxidizing action of a ferment upon the alcohol sorbitol which is contained in the juice.

REACTIONS OF THE CARBOHYDRATES.

The aldehyde and ketone, or *potentially*[1] aldehyde and ketone structure of the sugars renders them peculiarly liable to oxidation. Like other aldehydes and ketones, they reduce metallic oxides in alkaline solution; thus they reduce cupric to cuprous oxide, upon which fact **Fehling's method** of sugar-estimation is based, and they reduce silver salts in ammoniacal solution, leading to the formation of a silver mirror. Other reactions which are characteristic of the sugar-group are the following:

On heating a solution of sugar in concentrated sodium or potassium hydroxide, the liquid turns dark brown (**Moore's test**).

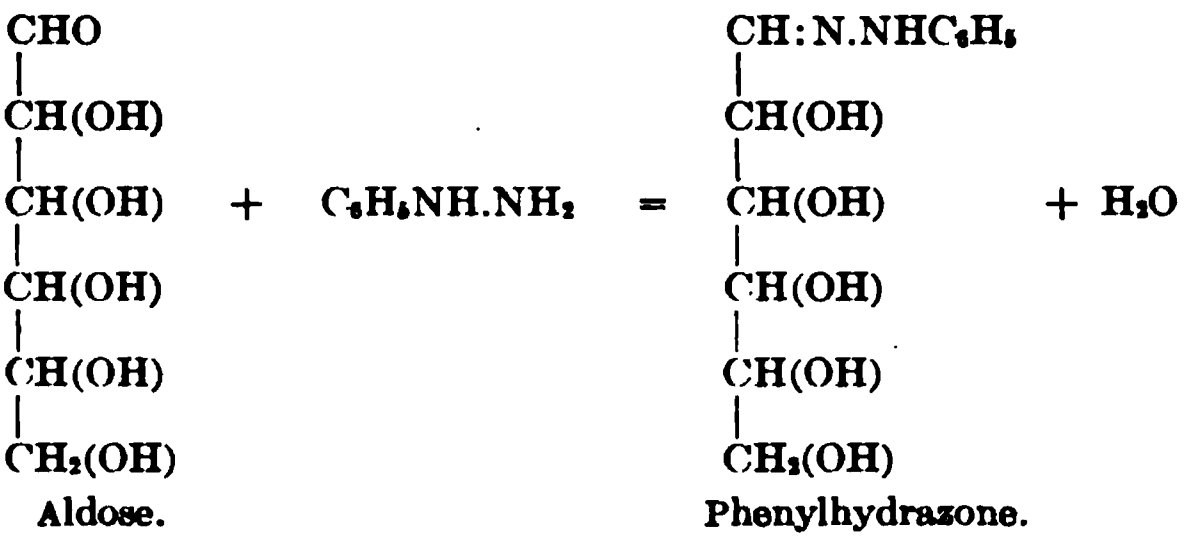
If to about 0.5 c.c. of a dilute aqueous solution of glucose are added a few drops of a ten per cent. alcoholic (acetone free) solution of α-naphthol, and 1 c.c. of concentrated sulphuric acid be cautiously run into the lower part of the tube, so that the lighter solution floats upon the top of the heavy acid, the zone of contact becomes reddish violet (**Molisch's test**). This reaction is due to the formation

[1] Cf. below.

of furfurol from the sugar by the concentrated acid. The furfurol then reacts with the α-naphthol yielding a colored product.

If sugar be heated to a considerable degree the mass partially carbonizes and turns deep brown. Numerous products are formed to which the collective name of **caramel** is given. Caramel has distinctively colloidal properties and very high coloring-power, upon which depends its use in the artificial coloring of beverages.

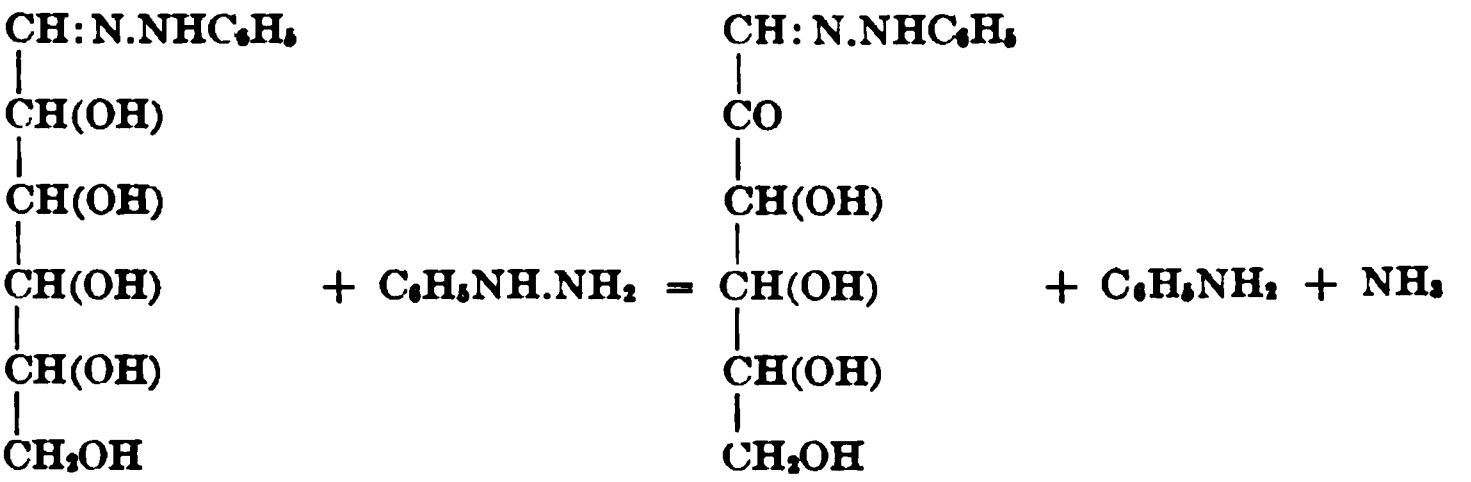
The sugars themselves are very soluble and on that account are frequently difficult to characterize and to purify. They form, however, insoluble or sparingly soluble compounds with **Phenylhydrazine**, which are of great service in characterizing the various sugars, enabling us to identify them in many cases with a considerable degree of certainty. If an aldose, or sugar, that is, of the aldehyde type, be acted upon by phenylhydrazine in the presence of excess of acetic acid, the following reaction occurs:



Aldose. Phenylhydrazone.

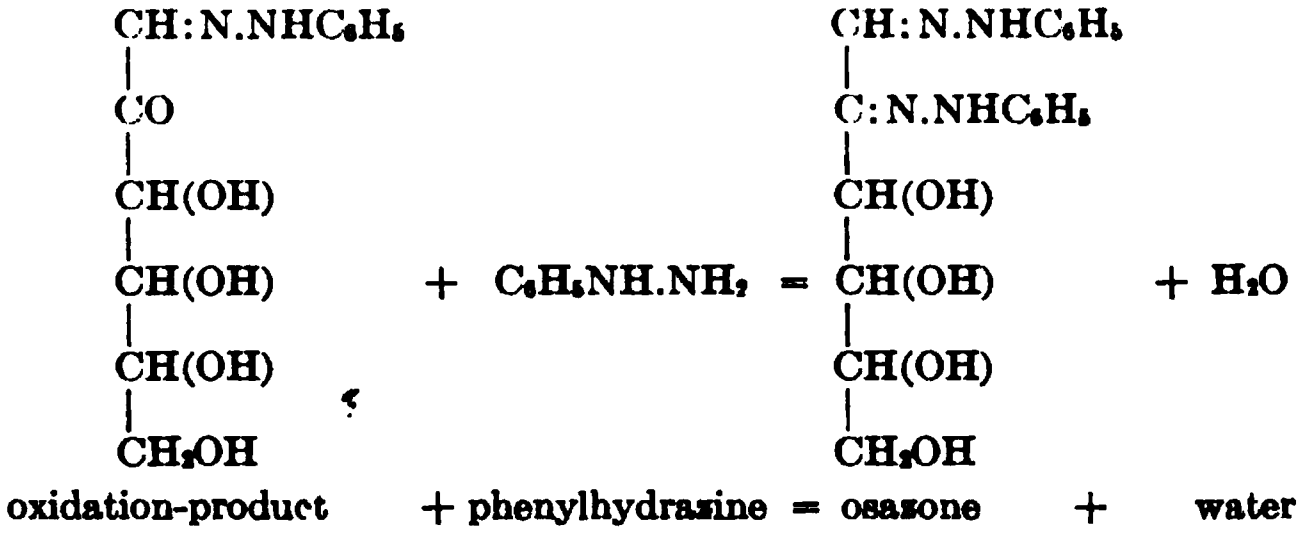
The **Phenylhydrazones** are, for the most part, readily soluble; so that this stage of the reaction is easily overlooked, since the subsequent secondary reactions which are about to be described produce sparingly soluble substances. Mannose is, however, an exception to this rule, the phenylhydrazone being very sparingly soluble and readily detected and isolated. Other hydrazines such as methylphenylhydrazine, benzylphenylhydrazine, and diphenylhydrazines also react with sugars to form hydrazones which are in some cases sparingly soluble and can readily be separated and purified by repeated recrystallization, and identified by their melting-points.

If excess of phenyl hydrazine be employed, the remainder of the reagent which is not used up in converting the sugar into a hydrazone acts as an oxidizing agent, converting a $-CH(OH)$ group into a $-CO$ group, thus:



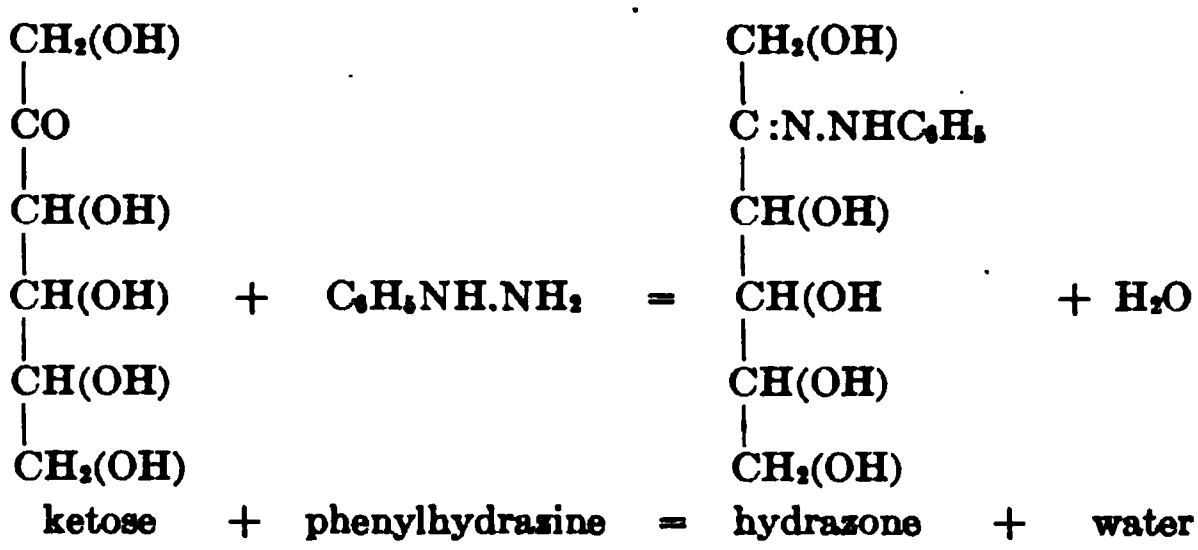
Hydrazone + phenylhydrazine = oxidation product + aniline + ammonia.

This oxidation-product subsequently reacts with yet another molecule of phenylhydrazine, with the formation of an **Osazone**:

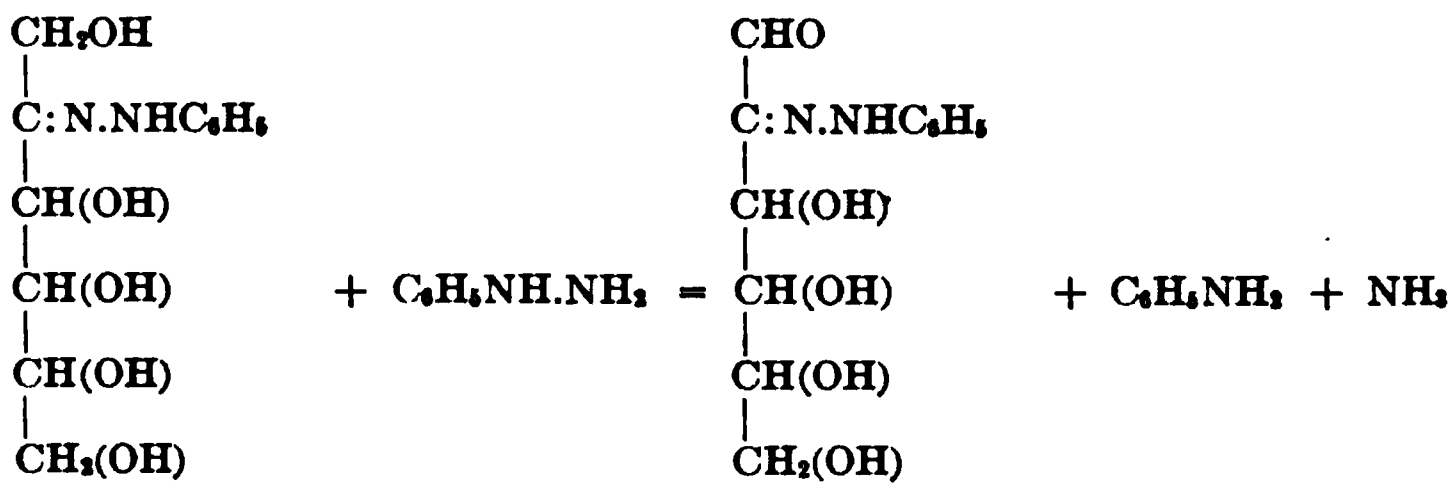


oxidation-product + phenylhydrazine = osazone + water

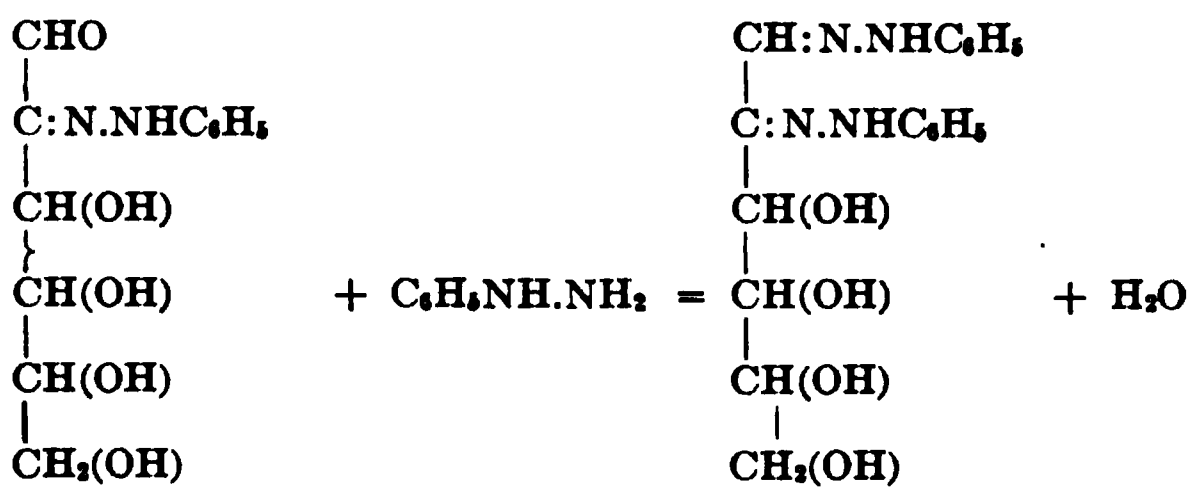
Glucose, mannose and fructose all yield the same osazone. In the case of fructose the reactions described above simply take place in the reverse order, thus:



ketose + phenylhydrazine = hydrazone + water



hydrazone + phenylhydrazine = oxidation-product + anilin + ammonia



oxidation-product + phenylhydrazine = osazone + water

The osazones as a class are characterized by their relatively slight solubility in water. They form yellow needle-shaped crystals and the shapes of the individual crystals and the way in which they group

C.

FIG. 1.—Osazone crystals. *A*, phenylglucosazone; *B*, phenylmaltosazone; *C*, phenyllactosazone. (After Halliburton.)

together are to some extent characteristic for each osazone. The melting-points of the osazones are not very definite, depending somewhat upon the mode in which the heat is applied, but they are, as a rule, sufficiently definite to serve as a means of identification.

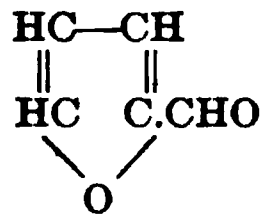
When the **Hexoses** are heated for prolonged periods with dilute mineral acids (excepting nitric acid) they yield **Levulinic Acid** (acetyl propionic acid) and formic acid, besides "humin substances" containing a higher proportion of carbon than the carbohydrates. The reaction producing levulinic acid proceeds as follows:

$$\underset{\text{hexose}}{C_6H_{12}O_6} = \underset{\text{levulinic acid}}{CH_3CO.CH_2.CH_2.COOH} + \underset{\text{formic acid}}{H.COOH} + \underset{\text{water.}}{H_2O}$$

When nitric acid is employed **Saccharic Acid** is produced (see below).

Levulinic acid is soluble in water, alcohol and ether and forms a colorless viscous liquid which boils at 250° C. and yields with silver nitrate a crystalline salt with the formula $CH_3.CO.(CH_2)_2COOAg$.

The **Pentoses** do not yield levulinic acid on boiling with mineral acids; on the contrary, they yield **Furfurol**



which may be collected by distillation and detected by the aid of aniline acetate paper, which is colored red by furfurol. This reaction may also be used for the estimation of pentose since the yield of furfurol is quantitative. For this purpose the furfurol is distilled and bisulphite added to the distillate, when the usual bisulphite compound with aldehydes is formed and the unconsumed bisulphite is estimated by titration with iodine. Or the furfurol may be converted into the phloroglucide by addition of phloroglucin and the yield of this compound determined gravimetrically. It should be very carefully borne in mind, however, that **Glucuronic Acid** and its compounds (see below) also yield furfurol on treatment with dilute acids so that before deciding that pentoses are present their identity should be established by the formation of osazones and the absence of glucuronic acid.

The pentoses also yield the following reactions:

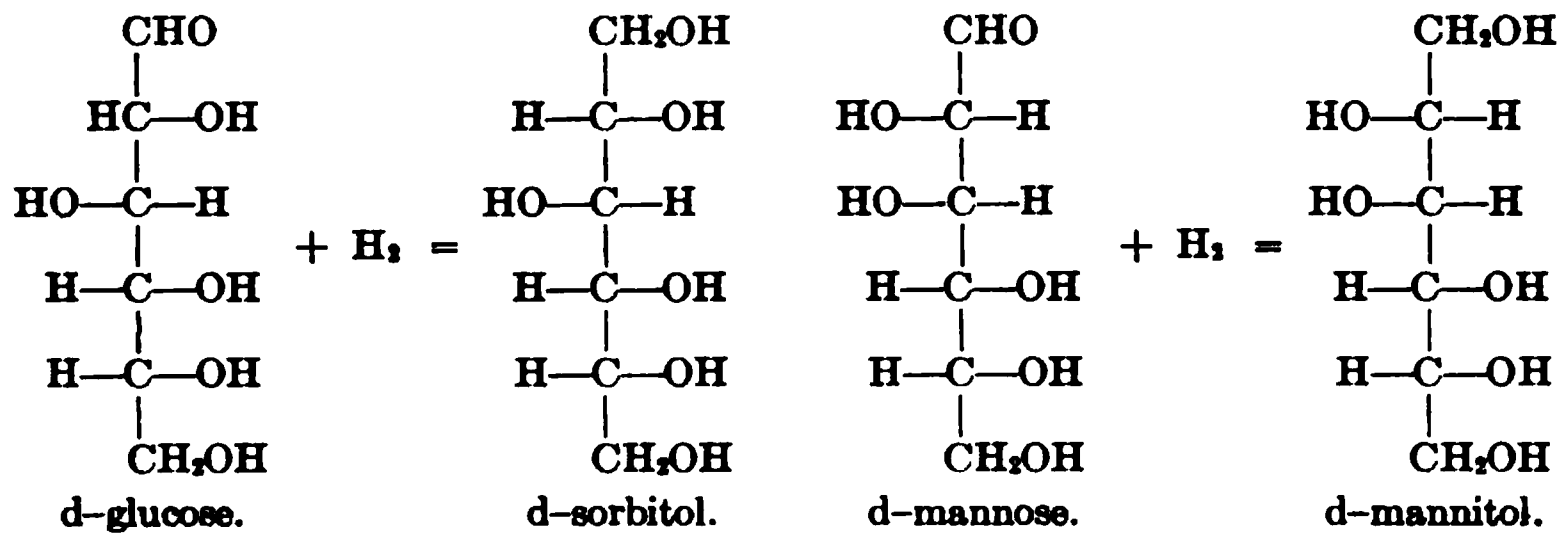
The Orcin Reaction.—To a small amount of the solution is added an equal volume of a solution of orcin in concentrated hydrochloric acid. On heating, the solution turns reddish-blue and then bluish-green. If pentoses are present in abundance, a green precipitate will be obtained on cooling and standing. On shaking up the mixture with amyl alcohol the green coloration passes over into this solvent. This reaction is also yielded by glucuronic acid.

The Phloroglucin Reaction.—This reaction is carried out in the same manner as the above, phloroglucin being used in the place of orcin. The mixture turns red on heating and becomes cloudy on cooling On shaking with amyl alcohol the red color passes over into the amyl. alcohol layer. This reaction is also yielded by glucuronic acid.

Selivanoff's Reaction.—The **Ketoses** may be distinguished from the **Aldoses by Selivanoff's reaction,** as follows:

To a few cubic centimeters of solution is added an equal volume of twenty per cent. solution of hydrochloric acid containing a small proportion of resorcinol. The liquid turns red on heating and a red substance is gradually deposited which is soluble in alcohol. This reaction depends upon the formation of oxymethyl-furfurol from the ketose by heating with acids. If the acid be too concentrated or the mixture boiled for more than about twenty seconds, the hexoses will also yield a small proportion of oxymethyl-furfurol and will in consequence yield the same reaction. It is necessary therefore to avoid a higher concentration of hydrochloric acid in the final mixture than about twelve per cent., and to boil only for a period of less than twenty seconds. This danger of confusion with the aldoses may be avoided by employing glacial acetic acid containing a small proportion of hydrochloric acid, instead of concentrated hydrochloric acid, as the solvent for the resorcinol.

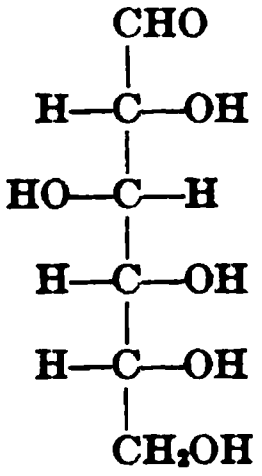
The Chemical Relationships of the Sugars.—Each sugar being an aldehyde or ketose, or at any rate *potentially* an aldehyde or ketose, corresponds to an alcohol from which it is derived by oxidation. Reduction of the sugars, therefore, results in the formation of alcohols. Glucose yields **Sorbitol**, mannose yields **Mannitol** and galactose yields **Dulcitol**. The following are the formulæ which illustrate the structure of these alcohols and their derivation from the corresponding sugars.

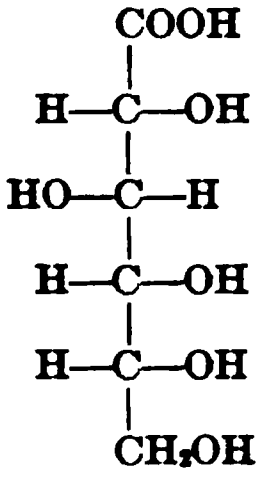


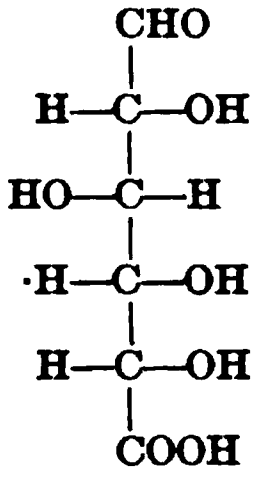
All of these alcohols occur in plants, mannitol especially being widely distributed. They have a sweet taste, but they are not fermentable by yeast.

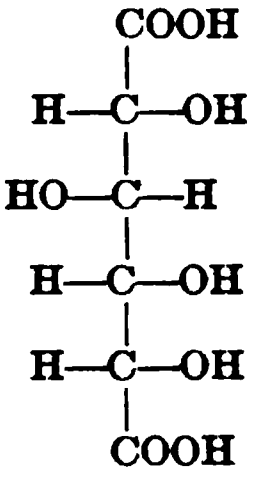
Just as the sugars, being potential aldehydes or ketoses, are con-

verted by reduction into **Alcohols**, so, by oxidation, they are converted into acids. Glucose yields three different 6-carbon atom acids on oxidation. Two of these acids are monobasic and the third is dibasic.


Glucose.


Gluconic acid.


Glucuronic acid.


Saccharic acid.

From mannose the dibasic acid which is obtained is **Manno-saccharic Acid.** From galactose the dibasic acid which results from oxidation is **Mucic Acid.** The ketoses, including levulose, behave quite differently on oxidation. The aldoses, on being oxidized yield acids containing the same number of carbon atoms as the original sugar. The ketoses, on the contrary, break down on oxidation and yield compounds containing fewer carbon atoms than the original sugar.

Similar relationships subsist among the sugars which contain fewer than six carbon atoms. Thus we have:

BIOSES.

Alcohol.	Sugar.	Monobasic acid.	Dibasic acid.
CH_2OH	CHO	COOH	COOH
|	|	|	|
CH_2OH	CH^2OH	CH_2OH	COOH
Glycol.	Glycolose.	Glycollic acid.	Oxalic acid.

TRIOSES.

Alcohol.	Sugar.	Monobasic acid.	Dibasic acid.
CH_2OH	CHO	COOH	COOH
|	|	|	|
CHOH	CHOH	CHOH	CHOH
|	|	|	|
CH_2OH	CH_2OH	CH_2OH	COOH
Glycerol.	Glycerose.	Glyceric acid.	Tartronic acid.

TETROSES.

CH_2OH	CHO	COOH	COOH
|	|	|	|
CHOH	CHOH	CHOH	CHOH
|	|	|	|
CHOH	CHOH	CHOH	CHOH
|	|	|	|
CH_2OH	CH_2OH	CH_2OH	COOH
Erythritol.	Erythrose.	Erythric acid.	Tartaric acid.

Similarly, the pentose **Arabinose** corresponds to the alcohol **Arabitol** and to the acids **Araboric** and **Trioxyglutaric**, while the pentose **Xylose** corresponds to the alcohol **Xylitol.**

By appropriate methods it is possible to convert sugars into others containing more carbon atoms and *vice versa*. Thus the aldoses combine directly with **Hydrocyanic Acid** with the formation of **Nitriles**, in accordance with the equation:

$$C_5H_{11}O_5.CHO + HCN = C_5H_{11}O_5CH(OH).CN$$

These nitriles, on hydrolysis, yield acids containing one carbon atom more than the original carbohydrates, thus:

$$C_5H_{11}O_5.CH(OH).CN + 2H_2O = C_5H_{11}O_5CH(OH)COOH + NH_3$$

Reduction of these acids, by means of sodium amalgam, yields the corresponding aldose with one carbon atom more than the original sugar. In this way glucose has been prepared from arabinose, and seven- and even nine-atom sugars have also been prepared, by successive steps, starting with glucose.

The conversion of a sugar containing more, into one containing fewer carbon atoms may be accomplished by converting the sugar by gentle oxidation into the corresponding (monobasic) acid, and then subjecting the calcium salt of this acid to further oxidation, with the result that the carboxyl-group is decomposed into carbon dioxide and water, and a sugar containing one less carbon atom than the original sugar is formed:

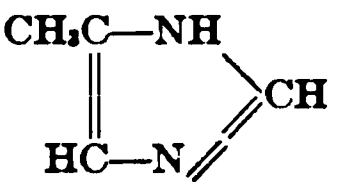
CHO	COOH	
CH(OH)	CH(OH)	COH
CH(OH)	CH(OH)	CH(OH)
CH(OH)	CH(OH)	CH(OH) + CO_2 + H_2O
CH(OH)	CH(OH)	CH(OH)
CH_2(OH)	CH_2(OH)	CH_2(OH)
Aldo-hexose	Aldonic acid	Aldo-pentose

This reaction is of very great interest to the biochemist because the conversion of a carboxyl-group into CO_2 and H_2O is known to be readily accomplished by bacterial action and probably also by animal tissues. The possibility is thus indicated that pentoses in the tissues may be derivable from glucose, a possibility, the significance of which will be apparent at a later stage.

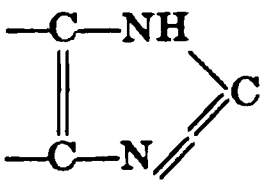
Not only is it possible to convert a hexose into a pentose and *vice versa*, but it is also possible to convert one hexose into another. It was found by Lobry de Bruyn that in the presence of alkalies, glucose, mannose or levulose in aqueous solution yields a mixture of the three sugars. More concentrated alkali brings about more pronounced decomposition, as is evidenced by the formation of lactic acid and other hydroxy-acids in Moore's test for carbohydrates. The production of lactic acid from glucose by the action of alkalies is a phenomenon of

great importance in the light of the fact that the decomposition of glucose, or glycogen which is an anhydride of glucose, in muscular tissue leads to the formation of lactic acid.

The action of alkalies upon glucose led to the suspicion that if ammonia were employed amino-derivatives of hydroxy-acids, such as are found among the constituent radicals of the proteins, might possibly be formed, and it was found by Windaus and Knoop that, as a matter of fact, ammonia, acting upon glucose, mannose, levulose, sorbose, arabinose, xylose, rhamnose or lactose yields **Methyl Glyoxaline**:



No other amino-products of this decomposition were identified, but this one is of extraordinary interest, because of the very great importance and variety of roles played by the **Iminazole** ring in physiological

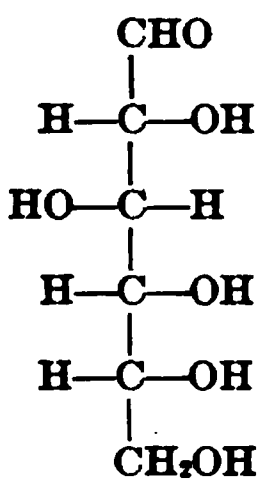


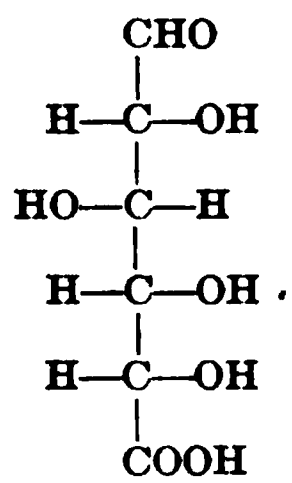
phenomena. While it is very doubtful whether the synthesis of this ring is possible for animal tissues to accomplish, and in fact there is much evidence tending to show that it is not, yet it is, of course, unquestionably accomplished by vegetable tissues, since the iminazole ring in the form of the amino-acid **Histidine** and in the purine-base moiety of the **Nucleic Acids**, is an invariable and essential constituent of living matter.

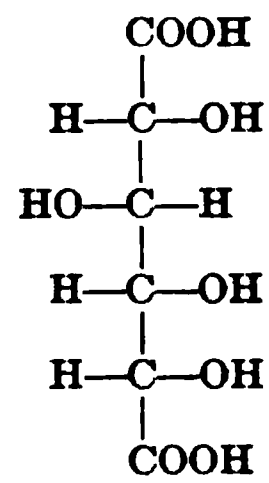
On heating hexoses in concentrated solution with amino-acids (glycocoll, alanine, leucine, tyrosine or glutamic acid) the mixture darkens with the formation of "**Humin Substances**" which are very deeply colored. At the same time carbon dioxide is discharged from the mixture. It is believed that the carbon dioxide is released from the carboxyl-group of the amino-acid which unites with the aldehyde-group of the sugar to form cyclic compounds. Similar substances are formed (from tryptophane) when proteins are hydrolyzed by strong acids in the presence of carbohydrates.

Certain Derivatives of Glucose.—Two derivatives of glucose, **Glucuronic Acid** and **Glucosamin**, claim our attention at this juncture, because they are both of profound physiological importance.

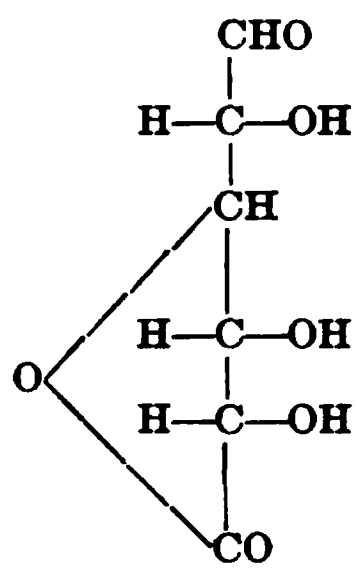
We have seen that on oxidation, glucose yields, first two monobasic acids and thereafter, on continued oxidation, each of these monobasic acids yields the same dibasic acid. One of the monobasic acids is glucuronic acid, the dibasic acid is saccharic acid. The connection between glucose, glucuronic acid and saccharic acid can be seen at a glance from their formulæ:


d–glucose.


d–glucuronic acid.


d–saccharic acid.

Glucuronic acid is therefore at the same time an acid and an aldehyde. On boiling its solution or on prolonged standing it is transformed into a crystalline lactone which is represented by the formula:



Glucuronic acid yields the pentose reactions with orcin or phloroglucin and hydrochloric acid (see p. 62) and also the following reaction:

Naphtho-resorcinol Reaction.—A small amount of naphtho-resorcinol is dissolved in concentrated hydrochloric acid and to this reagent is added an equal volume of a solution of glucuronic acid. A violet-blue coloration results which is soluble in ether. This reaction is not specific for glucuronic acid, being given by many ketose and aldehyde acids. It is, however, useful for the purpose of distinguishing between glucuronic acid and the pentoses.

Glucuronic acid does not occur in the free condition in animal tissues, nor has it as yet been identified in plants. In the form of ester-like compounds, however, it is found in many plants, notably in *Scutellaria*, and esters of glucuronic acid are found in many parts of the body, in the blood, the liver and in urine. The normal forms in which it is found in urine are **Phenyl-**, **Indoxyl-** and **Skatoxyl-glucuronic** acids. Indoxyl and skatoxyl are highly toxic products of intestinal putrefaction; the compounds which they form with glucuronic acid are, however, harmless.

Under ordinary conditions, glucose is readily oxidized in the body to carbon dioxide and water, passing through intermediate stages of which lactic acid is one. But in the presence of some toxic agents it appears that the oxidation of glucose is arrested at the formation of glucuronic acid, which combines with the toxic substance, the compound being

eliminated as such. It is not known whether or not glucuronic acid is a normal intermediate product of glucose oxidation in the animal body, or whether it is only formed under the exceptional condition of the presence of certain toxic bodies. Large quantities of glucuronic acid, in these ester-like combinations, appear in the urine when certain drugs are introduced into the system. The following is a partial list of the drugs which are eliminated in this manner:

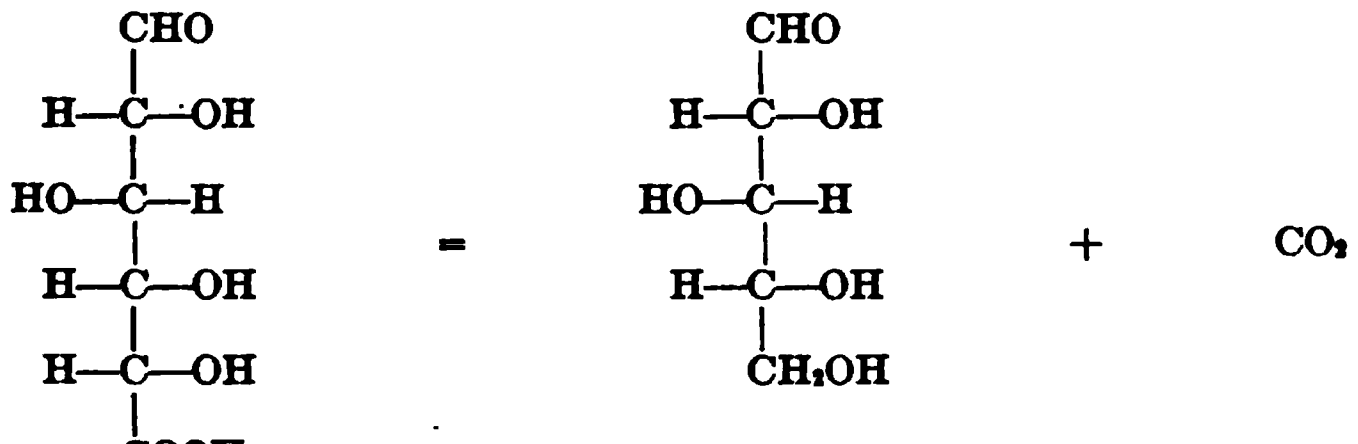
Isopropyl alcohol	chloral
Methyl propyl alcohol	butyl chloral
Methyl ethyl carbinol	bromal
Tertiary butyl alcohol	dichloracetone
Tertiary amyl alcohol	α and β napthol
Benzol	turpentine
Nitrobenzol	camphor
Aniline	borneol
Phenol	menthol
Resorcinol	pinene
Thymol	antipyrine

The elimination of these drugs in this manner constitutes a pitfall for the unwary who may seek, after the administration of such drugs as these to a patient, to investigate the urine for the presence of sugar therein by the phenylhydrazine test, or its clinical modification known as **Cipollina's test.** For glucuronic acid forms an osazone which may easily be mistaken for glucosazone. The distinction may very readily be made, however, owing to the fact that the osazone of glucuronic acid is decomposed by heating while that of glucose is not. If the precaution be taken, therefore, of heating the precipitate on a boiling water-bath for half an hour before examining it, no confusion of the osazone of glucuronic acid with glucosazone is possible, for the osazone of glucuronic acid is decomposed by this procedure and redissolves, while the osazone of glucose remains unaltered.

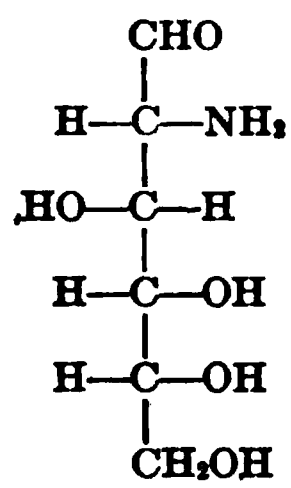
Glucuronic acid is therefore to be regarded as a protective agent which guards the organism against the deleterious action of certain substances introduced from without or, in some cases, from within the body. Sometimes the glucuronic acid accomplishes its protective function by combining directly with the toxic substance, rendering it harmless until in the course of time it is eliminated; in other instances the toxic substance undergoes some degree of change and elaboration before it is paired with glucuronic acid. Thus chloral hydrate and butyl chloral undergo reduction before they couple with the glucuronic acid; o-nitrotoluol, on the contrary, is oxidized to nitrobenzyl-alcohol before it pairs with glucuronic acid. Other substances undergo hydration or both hydration and oxidation before they can couple with the glucuronic acid.

Glucuronic acid is possibly of importane not only as a carrier of toxic substances out of the body, but also as a oonnecting-link between the hexoses and the pentoses. It will be recollected that when a mono-

basic acid derivative of an aldohexose is acted upon by oxidizing agents, the carboxyl group is eliminated in the form of carbon dioxide and water, and the corresponding aldo-pentose is formed. When d-glucuronic acid is subjected to intense putrefaction, it undergoes an analogous change, with the production of l-xylose, thus:



Glucosamin, on the other hand, affords a connecting link between the carbohydrates and the hydroxy-amino-acids. It is readily obtained in considerable quantities from the exoskeletons of *Crustacea*, as for example from the shells of lobsters, by boiling with concentrated hydrochloric acid. It also occurs in fungi and it is a constituent of the **Mucins** and **Mucoids**; sticky glutinous proteins which are found in mucous secretions and elsewhere. The formula of glucosamin is:



In the true mucins, but not in the mucoids the glucosamin radical is probably acetylated, and acetyl glucosamin, in common with other acetyl derivatives of hydroxy-amino-acids, yields **Ehrlich's Reaction**, namely a pink color when its solution is mixed and warmed or allowed to stand with an equal volume of a two per cent. solution of paradimethylaminobenzaldehyde in hydrochloric acid of specific gravity 1.09. The mucins also yield this reaction.

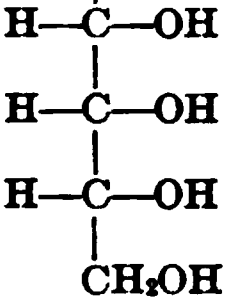
THE DISTRIBUTION OF THE MONOSACCHARIDES IN LIVING TISSUES.

As has been stated, a pentose, **d-Ribose** is a normal constituent of the nucleoproteins. The following, after Grund, is the percentage

of pentoses calculated on the basis of the dry tissue, which is present in various parts of the mammalian body:

Pancreas	2.48
Liver	0.56
Thymus	0.56
Submaxillary gland	0.53
Thyroid gland	0.50
Kidneys	0.49
Spleen	0.46
Brain.	0.22
Muscle	0.11

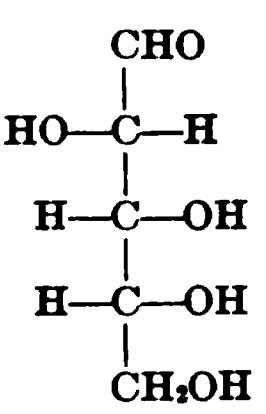
The structural formula of d-ribose may be represented as:



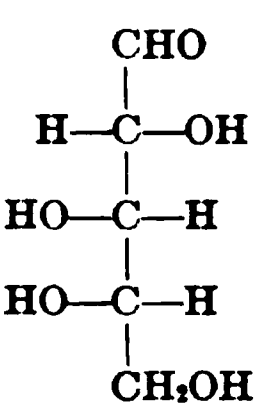
d-ribose.

it is levorotatory, the prefix d- being employed to denote its relationship to d-altose and d-altrose.

In certain very exceptional cases a pentose is formed in the urine. The disease which leads to this elimination of pentoses is known as **Pentosuria**, in contradistinction to **Glycosuria**, the very much more common elimination of glucose. Only a few cases of pentosuria have been observed, but it is an extremely noteworthy fact that the pentose which is eliminated in this disease would appear to be almost invariably optically inactive, although the pentose, l-ribose, which is normally found in the tissues is, of course, optically active. Not only this, but the pentose in the urine is not ribose but **Arabinose**,



d-arabinose.



l-arabinose.

which would seem to point to its derivation from glucose rather than from the decomposition of nucleo-proteins, for it will be remembered that arabinose may be derived from glucose by the oxidation of the calcium salt of gluconic acid (xylose being the corresponding pentose resulting from the oxidation of glucuronic acid). However this may be, the pentose elimination in these cases is independent of the pentose-

content of the food and may occur when the combustion of carbohydrates in the tissues would appear to be otherwise normal.

The pentoses are widely distributed in the vegetable kingdom, chiefly in the form of polysaccharides, bearing the same relation to the pentoses as starch and glycogen do to the hexoses. These polysaccharides, which are complex anhydrides of the monosaccharides, are known as **Pentosans**. The following table shows the percentage of pentosan, in terms of pentose, found in the dry substance of various vegetable foods:

Meadow hay	21.64
Rape cake	11.50
Oil-seed cake	9.07
Bruised barley	7.96
Rice flour	5.73
Sesame cake	3.87
Table turnip	1.13
Spinach	1.02

With regard to the distribution of the hexoses; **Levulose** is not often found in the animal kingdom. In honey it occurs together with glucose and is immediately derived from the juices of flowers, but it is a question whether it is ever normally found in animal tissues. It is occasionally found in the urine, and is then derived directly from the levulose absorbed from the intestine; it may be regarded as a sign either of excessive overindulgence in sweets or honey or else, if this origin can be excluded, as a sign of overactivity of the pituitary gland, which as we shall see later on, lowers the limit of tolerance for all forms of sugar. A urine which yields evidence of the presence of a reducing sugar should therefore always be tested for levulose by **Selivanoff's test** (see p. 62) before a provisional diagnosis of diabetes is decided upon.

In vegetable tissues levulose is widely distributed, especially combined with glucose to form cane-sugar. It is also found in the form of a complex anhydride, or polysaccharide, **Inulin** in the tubers of dahlias and in the sweet potato.

Grape-sugar, or d-glucose, is the most important of all the monosaccharides in the animal economy. It is the central figure in the carbohydrate metabolism. Polysaccharides are broken down to glucose before assimilation, and again before utilization as a source of energy, or transportation from one part of the body to another. It is the circulating form of carbohydrate, **Glycogen** and other polysaccharides being the storage-forms. In view of these facts the absurdity will be apparent of the effort which was made in certain circles in the United States, a few years ago, to represent glucose and glucose-syrups as deleterious articles of food. Provided they contain no other constituents which are harmful such preparations are merely solutions of the only carbohydrate which is to any important extent a normal and invariable constituent of the blood.

Normal urine contains minute traces of glucose, and sometimes larger amounts, especially after a meal which is very rich in carbohydrates. Such glycosuria is known as **Alimentary Glycosuria** and is devoid of significance unless it occurs too frequently and readily, in which case it may possibly indicate disturbance of the functions of the pituitary gland. In certain pathological conditions or under experimental conditions much profound and serious glycosurias may occur. These will fall under consideration in a later chapter.

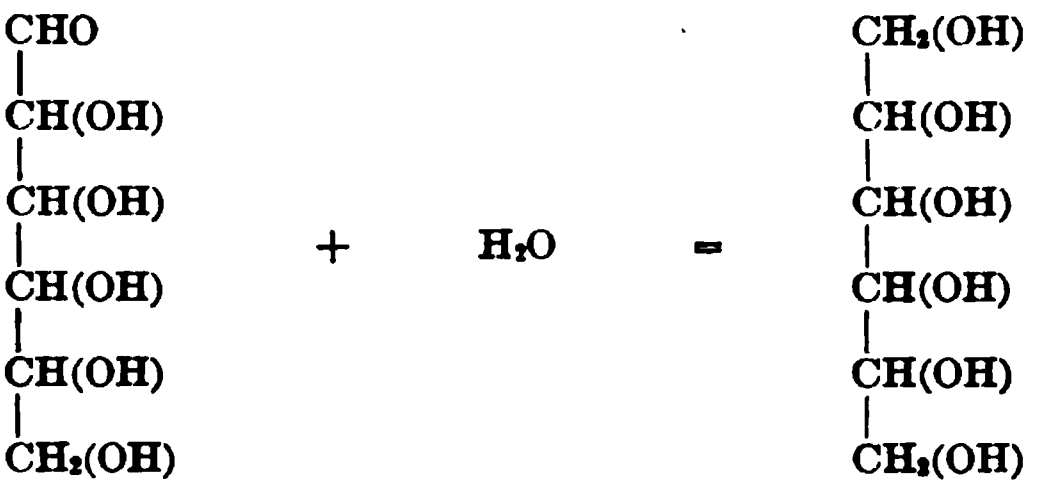
Galactose is found in important quantities in two places in the animal kingdom, namely combined with glucose to form milk-sugar or **Lactose**; and in the form of glucoside-like compounds, the **Cerebrosides**, which are found in the brain.

THE LACTONE-STRUCTURE OF SUGARS.

Before proceeding to the consideration of the disaccharides, it is important to review some recent accessions to our knowledge of the sugars which have led us to reconsider in some degree the structural formulæ by means of which we have hitherto represented them. It is necessary to enter thus deeply into the subject of the configuration of the sugar molecule because a clear understanding of these questions has already fundamentally contributed to our knowledge of the mode of action of ferments, and is unquestionably destined to do so even to a greater degree than heretofore. In considering the enzymatic hydrolysis and synthesis of the disaccharides we shall have occasion to refer very frequently to the facts which are about to be described.

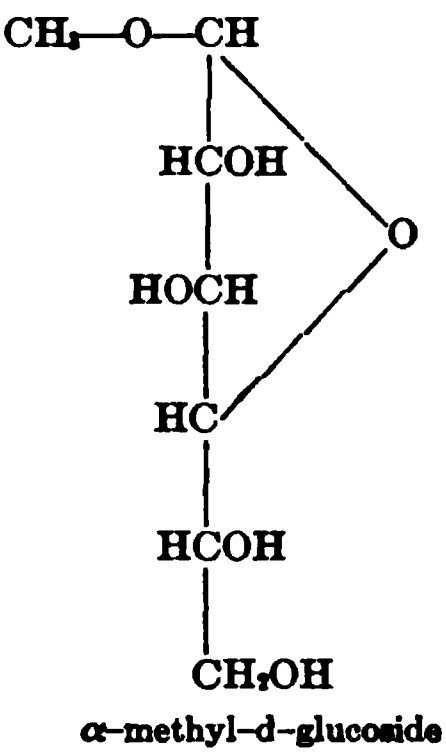
It will be recollected that in compounds containing only four asymmetric carbon atoms, such as we have been assuming the hexoses to be, only sixteen stereo-isomers are possible. Now, as a matter of fact, it has long been known to sugar-chemists that the optical rotatory power of solutions of d-glucose is not a constant quantity. The optical rotatory power of fresh solutions changes gradually, sometimes increasing, but more usually falling, until a constant value is ultimately reached. This constant value is the same for all glucose solutions which have attained equilibrium, but the initial rotatory power of fresh solution may be as much as twice as great as the final constant rotatory power. This phenomenon is variously known as **Mutarotation, Multirotation** and **Birotation.**

Analogous phenomena in other solutions are generally attributed to the presence of two or more different, optically active substances, of different rotatory power and convertible into one another. Adopting this point of view, Emil Fischer first suggested, in explanation of the phenomenon of mutarotation, that the glucose undergoes hydration in solution, with the formation of an alcohol of lower rotatory power, thus:

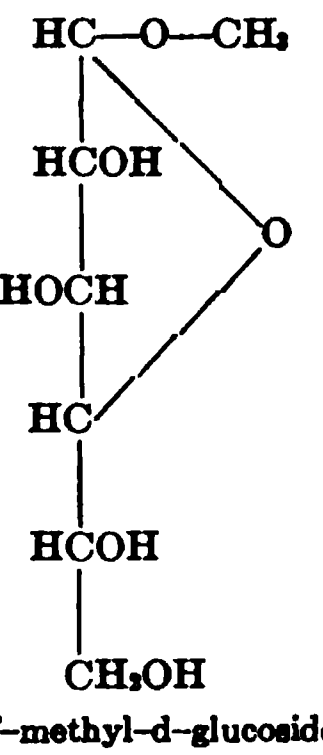


This view, which never had any experimental support, was rendered unnecessary and untenable by the discovery of the fact that two different forms of d-glucose are obtainable, isomers of one another but differing in rotatory power. The one form, α-d-glucose, crystallizes out at ordinary temperatures from seventy per cent. alcohol, and has a molecular rotation of $(\alpha)_D + 110°$; the other, β-d-glucose, crystallises out from solutions in water at temperatures above 98° C., and has a molecular rotation of $(\alpha)_D + 19°$. It appears that there are indeed two stereo-isomeric forms of d-glucose, which would be impossible were there only four asymmetric carbon atoms in the molecule, as the formula $CHO-CH(OH)-CH(OH)-CH(OH)-CH(OH)-CH_2(OH)$ requires. The glucose molecule must, in fact, contain not less than **Five** asymmetrical carbon atoms. This conclusion, first suggested by Simon, was verified by Armstrong in the following way:

Two methylated d-glucoses are known, formed from glucose by the replacement of a hydrogen by a methyl group. The structures of these two methyl glucosides are believed to be respectively:



α–methyl–d–glucoside

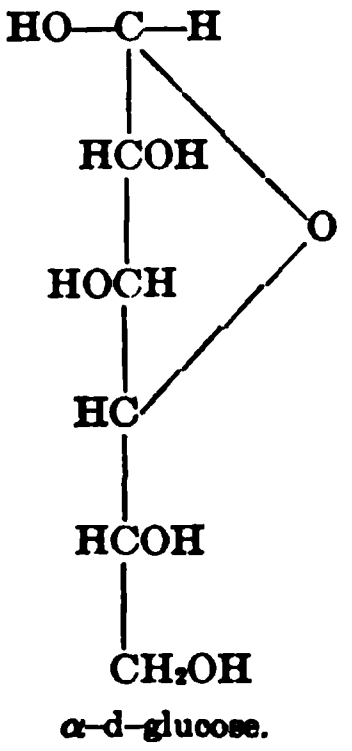


β–methyl–d–glucoside

Each of these glucosides can be hydrolyzed by an appropriate ferment. Now it is observed that a glucose of high rotatory power is produced in the hydrolysis of the α-methyl glucoside, while on adding a drop of ammonia to the solution the rotation rapidly falls to the equilibrium-value of the rotatory power of ordinary glucose. On the other hand, when the β-methyl glucoside is hydrolyzed, a glucose of low rotatory power is produced, and on adding a drop of ammonia to

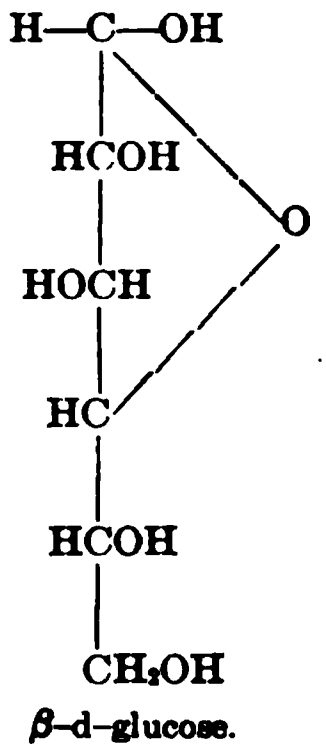
the solution the rotatory power rapidly rises to the equlibrium-value of the rotatory power of ordinary glucose.

From these observations it appears that the true formula for d-glucose is either:



α-d-glucose.

or



β-d-glucose.

of which the former is the α (highly rotating) form, and the latter the β form of low rotatory power. In solution, an equilibrium is finally attained between the two forms, and the attainment of this equilibrium is much accelerated by an alkaline reaction. The rotatory power of the pure α form is $(\alpha)_D + 110°$; that of the pure β form $(\alpha)_D + 19°$. The rotatory power of an equilibrated solution of the mixed glucoses is $(\alpha)_D + 52.5°$. From these figures it is a simple sum in proportion to calculate that in a ten per cent. solution of glucose, about thirty-seven per cent. is of the α form and about sixty-three of the β form at equilibrium.

We see that glucose contains, therefore, not four but five asymmetrical carbon atoms, a fact which is not revealed by a study of long-standing or equilibrated solutions and was therefore very naturally overlooked in the first attempts to attach a structural formula to individual hexoses. If this be true of the other hexoses as well, however, then there must exist not $2^4 = 16$ stereo-isomers of glucose, but $2^5 = 32$. As a matter of fact, we find that many of the sugars exhibit mutarotation, for instance d-glucose, d-galactose, d-mannose, d-fructose, l-arabinose, l-xylose, and some of the disaccharides. There is little room for doubt that the structural formulæ of each of these sugars are analogous to the formulæ for glucose which are depicted above.

Since the hexaldoses all give the aldehyde reactions, that is, reduce metallic oxides in alkaline solution, and unite with phenylhydrazine by means of an aldehyde group, we must suppose that in the presence of these reagents the oxide grouping is broken down and the aldehyde group regained. This fact is very readily understood if we suppose that every solution of glucose contains a trace of the aldehyde form, in equilibrium with the oxide forms. A reagent such as a metallic oxide or phenylhydrazine reacts with the trace of aldehyde form and thus

removes it from the solution; the oxide form is then no longer in equilibrium and therefore regenerates the aldehyde form in the process of regaining the equilibrium which has been disturbed. This fresh supply of the aldehyde form in its turn reacts and is removed from the solution, and so the process repeats itself until all of the sugar is used up. At the same time this view of the structure of the sugar enables us to understand why it is that although they give most of the aldehyde reactions, yet they give them much less energetically than the typical aldehydes.

We may also ascribe to the same source the fact that the sugars do not react in stoichiometrical proportions with metallic oxides; in the proportions, that is, which would be expected if a molecule of sugar reacted quantitatively with a molecule of metallic oxide. We cannot predict, by merely writing down chemical equations, how much of any metallic oxide under given circumstances will be reduced by a given amount of sugar. Instead, for every concentration of sugar employed and for every circumstance of the reaction, we have to estimate afresh, and by direct measurement, the reducing power of the sugar. These measurements are commonly expressed in tables which denote the relationship of reduced cupric oxide (or other metallic oxide) to the quantity of sugar present in the solution investigated. But such tables are empirically established and are therefore reliable only if the circumstances of concentration, reaction, temperature and so forth are exactly the same as those which prevailed in the estimations from which the tables were computed.

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CHAPTER IV.

THE CARBOHYDRATES: THE DISACCHARIDES, POLY-SACCHARIDES AND GLUCOSIDES.

THE DISACCHARIDES.

The disaccharides are carbohydrates which contain twelve carbon atoms, and are formed from two molecules of hexose with the elimination of water in accordance with the equation:

$$C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O.$$

The majority of the disaccharides reduce Fehling's solution (*i. e.*, cupric oxide in alkaline solution), react with phenylhydrazine to form hydrazones and osazones, and exhibit mutarotation in solution. They therefore contain a potentially aldehyde or ketone group or groups, and an oxide linkage analogous to that in glucose. Certain of them are exceptions to this rule, however, one of the most marked exceptions being cane-sugar, which is formed by the union of one molecule of glucose with one of fructose (levulose), and which does not reduce Fehling's solution nor react with phenylhydrazine, nor display mutarotation in solution.

The disaccharides are merely special instances of a very large group of compounds which are generically termed **Glucosides**, or compounds of sugars with other bodies, the point of union being the aldehyde group of the sugar. A typical glucoside, for example, is **Amygdalin**, found in cherry-stones and in almonds, which on hydrolysis yields glucose, hydrocyanic acid and benzaldehyde. The nucleic acids are glucosides. Glucosides which yield galactose on hydrolysis are found in the tissues of the brain. The disaccharides are glucosides in which both constituents of the molecule are sugars.

The most important disaccharides from the point of view of animal biochemistry are cane-sugar or **Sucrose, Maltose, Isomaltose, Lactose** and **Isolactose.** All of these excepting sucrose contain one potentially active aldehyde group; that is, they reduce Fehling's solution, form osazones and exhibit mutarotation.

Cane-sugar is the ordinary sugar of commerce and occurs widely distributed in the vegetable kingdom, where it acts as a reserve-material, that is, as a store of nutriment to be broken up into utilizable material and consumed when needed. It occurs especially in the sugar-cane, in the sap of certain palms and of the sugar maple, the birch and the carob tree. Ripe fruits and many leaves contain considerable amounts

of this sugar, while one of the most important sources of sugar is the root of the sugar-beet, a variety which has originated by selection from the common beet (*Beta maratima*). Cane-sugar was not known in Europe until its introduction from the tropical parts of Asia where the sugar-cane has been grown from time immemorial. The possibility of extracting cane-sugar from beets was not realized until it was pointed out by the German chemist Marggraff in 1760. Hence the large consumption of sugar now obtaining among European peoples is a recently acquired habit. It is, of course, of enormous nutritive importance as it results in reducing by an equivalent amount the requirement of starch and other polysaccharides. It also enables us, when sugar from the cane is used, to utilize tropical areas for the production of carbohydrate foodstuffs and set free greater areas of the temperate regions for the cultivation of polysaccharides and proteins (grains, meat and dairy products) for which the tropical areas of the world are not suitable. The consumption of sugar from the cane is therefore economically preferable to the consumption of sugar from the beet.

Cane-sugar does not reduce Fehling's solution nor does it exhibit mutarotation. It is neither potentially nor actually an aldehyde or a ketone. It is very readily hydrolyzed by acids, therein differing markedly from other disaccharides, and it yields on hydrolysis, one molecule of **d-Glucose** and one of **d-Fructose** (levulose). It will be recollected that d-fructose is levorotatory, and the levorotatory power of d-fructose being greater than the dextrorotatory power of d-glucose, the mixed products of cane-sugar hydrolysis are levorotatory. Cane-sugar, on the contrary, is dextrorotatory, so that hydrolysis of cane-sugar in solution leads to a change of optical rotation from right to left. Hence the process of the hydrolysis of cane-sugar is frequently termed **Inversion.**

Cane-sugar is built up by the union of a molecule of d-glucose with one of d-fructose. The question arises, however, from which of the two d-glucoses is cane-sugar derived; the α-d-glucose or the β-d-glucose? This question is answerable in a very simple way. It is possible to hydrolyse cane-sugar very much more rapidly than α-d-glucose can undergo transformation into β-d-glucose or *vice versa*. It will be recollected that α-d-glucose possesses a much higher dextrorotatory power than β-d-glucose. Now we find that the glucose produced in the hydrolysis of cane-sugar possesses, initially, a high rotatory power. On adding ammonia, which accelerates the transformation of α- into β-glucose, the rotation due to glucose falls. Hence the glucose set free in the hydrolysis of cane-sugar is α-glucose, and cane-sugar is therefore to be regarded as a derivation of α-d-glucose.

Cane-sugar does not react with phenylhydrazine. It contains eight hydroxyl groups, for it forms an octa-acetate, in which these groups have been replaced by acetyl groups. Apart from this it has not proved possible to ascribe any satisfactory constitutional formula to cane-sugar. The synthesis of cane-sugar has, however, been accom-

plished, by the interaction of potassium fructosate and acetochlor-glucose.[1]

Cane-sugar is not attacked by any ferments excepting **Invertase**, an enzyme found in many yeasts, moulds, and in some of the higher plants. Invertase, as its name implies, effects the hydrolysis of cane-sugar into its constituent parts, glucose and fructose; that is, it brings about "inversion." Cane-sugar, or, rather, its product, glucose, does not undergo alcoholic fermentation in the presence of yeasts until it is broken down into glucose and fructose. Hence yeasts which do not contain invertase are not able to cause alcoholic fermentation in solutions of cane-sugar.

Maltose is a disaccharide which results from the hydrolysis of starch or of glycogen by acids or by ferments. Acids, however, continue the process of hydrolysis by splitting the maltose itself, so that maltose is only a transient stage in the hydrolysis of starch or glycogen by acids. On the other hand the ferments which split starch or glycogen do not hydrolyze maltose, so that if maltose-splitting ferments be absent the process of hydrolysis ceases at this stage.

Maltose is highly dextrorotatory, exhibits, mutarotation, reduces Fehling's solution and forms a phenylosazone. When hydrolyzed by acids it yields two molecules of glucose, but it is much less readily hydrolyzed by acids than cane-sugar. The ferment **Diastase**, which hydrolyzes starch and glycogen, the ferment **Invertase** which hydrolyzes cane-sugar, the ferment **Lactase** which hydrolyzes milk-sugar, and the ferment **Emulsion** which hydrolyzes amygdalin and isomaltose, are all without action upon maltose, which is hydrolyzed only by a ferment known as **Maltase**, found in many animal tissues and in the majority of yeasts. Maltose itself does not undergo alcoholic fermentation, and must first be split by maltase or by acids into glucose, but as the majority of yeasts contain maltose, these yeasts can accomplish the reduction of alcohol from maltose.

The glucose which maltose yields upon hydrolysis is initially highly rotatory; on adding ammonia the rotation falls. Hence maltose is a derivative of α glucose. It is, in fact, glucose-α glucoside. It can, of course, exist in two forms, according to whether the glucose moiety which still contains a potential aldehyde group is in the α or β form. The one maltose, α-maltose, is therefore α-glucose-α-glucoside; the other is β-glucose-α-glucoside.

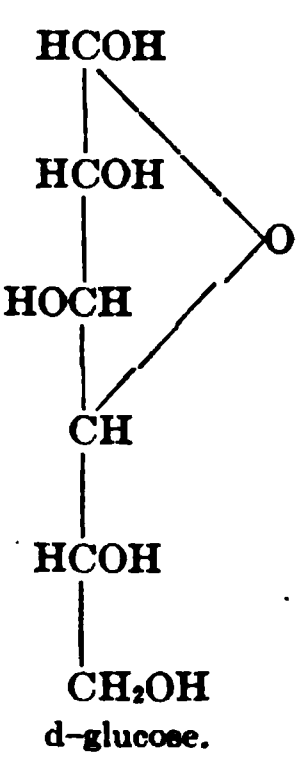
Maltose can be synthesized from glucose by the condensing action of strong acids. But in addition to maltose another disaccharide is obtained by this process. This disaccharide is isomeric with maltose and yields, like maltose, two molecules of glucose on hydrolysis. It differs from maltose in the characteristics of its phenylosazone, and also in the fact that it is not fermentable by yeasts. The ferment maltase, in fact, has no action upon it, while the ferment emulsion,

[1] Marchlewski in 1899.

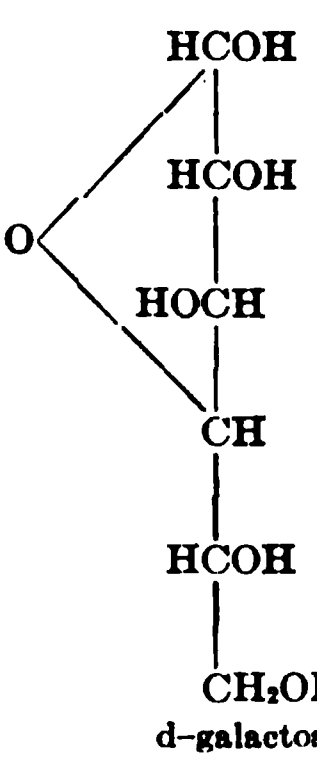
which is found in certain plant-tissues and which has no action upon maltose, hydrolyzes this sugar with the production of two molecules of glucose. This glucose, unlike the glucose which is produced in the hydrolysis of maltose, is of low initial rotatory power. On adding a drop of ammonia to its solution the rotatory power *increases*. Hence, this sugar, which is called **Isomaltose**, is a derivative of β-glucose. It is a mixture of α-glucose-β-glucoside, and β-glucose-β-glucoside.

Milk-sugar, also called **Lactose**, has not been encountered in the vegetable kingdom. It does not occur preformed in any item of our diet excepting in milk, nor does it appear likely that one of its constituent hexoses, galactose, is commonly obtainable from any other dietary source than milk. Of course it might be obtained from brain-tissue, but this cannot be regarded as a customary item of our dietary. Lactose yields, on hydrolysis, one molecule of d-glucose and one of d-galactose. Lactose exhibits mutarotation, reduces Fehling's solution, and forms a phenylosazone. Lactose is not hydrolyzed by maltase, invertase, diastase or emulsin, but it is hydrolyzed by a specific ferment designated **Lactase**, and found in the gastric mucous membrane and in a few yeasts such as Kephir yeast. This yeast is employed by the Arabs to make a sparkling alcoholic beverage, "Kephir," from the milk of mares.

Milk-sugar is found in varying amounts in the milk of all mammals. During pregnancy it is often found in small quantities in the urine; and after weaning it also tends to escape for a few days through the kidneys. Extirpation of the mammary glands in milch-goats and cows gives rise to a notable increase in the amount of sugar in the blood (**Glucohemia**) and also to the appearance of glucose in the urine (**Glycosuria**). It thus appears probable that in the mammary glands milk-sugar is formed from glucose alone, and not from glucose and galactose in the diet. On comparing the formulæ of glucose and galactose:



d-glucose.



d-galactose.

it will be seen that the transformation of galactose into glucose involves the rupture of the oxide-ring and its closure again on the opposite side.

No enzyme has yet been isolated which is capable of bringing about this transformation.

It is for this reason and possibly also for others connected with the metabolism of the intestinal bacteria, that maltose or cane-sugar cannot be regarded as satisfactory substitutes for milk-sugar in the diet of young infants. On the other hand, the assimilation-limit or quantity which may be ingested at once without leading to alimentary glycosuria, is lower for lactose than for the other sugars, so that if large quantities of sugar have to be given to make up the requisite calorific value of the diet, as in the case of fat-intolerant infants, maltose may be used as an accessory to lactose in the food. Cane-sugar being the disaccharide which is most foreign to animal tissues, and also the sweetest in taste, is much less suitable than milk-sugar or maltose for the dietary of young infants.

The galactose which milk-sugar yields on hydrolysis is of low rotatory power, and its rotation increases on adding ammonia. Hence lactose is glucose-β-galactoside, since it can be shown by forming the osazone of the sugar and hydrolyzing, when the phenylhydrazine remains attached to the sugar with the free (potential) aldehyde group, that it is the glucose radical which contains the potential aldehyde-group, the aldehyde-group of the galactose offering the point of union for the glucose molecule. The potential aldehyde-group of the glucose radical can exist either in the α- or the β-form. The α-lactose (rotatory power $= + 86°$) is therefore α-glucose-β-galactoside, while β-lactose (rotatory power $= + 35°$) is β-glucose $=$ β-galactoside. No derivative of α-galactose is certainly known to occur in nature. If, however, kephir lactase be allowed to act upon a *concentrated* mixture of equal parts of glucose and galactose, two isomeric lactoses are produced, both exhibiting mutarotation, and both yielding d-glucose and d-galactose on hydrolysis. One of these is ordinary lactose, the other has been termed **Isolactose** and is possibly a mixture of α- and β-glucose-α-galactosides.

Each of the disaccharides which contains a potentially active aldehyde-group can, therefore, exist in four different forms. Thus for maltose we have:

α glucose-α-glucoside	α-	maltose
β glucose-α-glucoside	β-	
α glucose-β-glucoside	α-	isomaltose
β glucose-β-glucoside	β-	

and for lactose we have:

α glucose-α-galactoside	α-	isolactose
β glucose-α-galactoside	β-	
α glucose-β-galactoside	α-	lactose
β glucose-β-galactoside	β-	

These relationships are very important, and we shall have occasion to refer to them again in later chapters.

Melibiose is a galactoside of glucose. It is derived from the trisaccharide raffinose by hydrolysis.

POLYSACCHARIDES.

We must now take up the consideration of the **Polysaccharides**, or carbohydrates formed by the union of more than two molecules of the simple sugars, with the elimination of a corresponding number of molecules of water.

A few tri- and tetra-saccharides are tolerably well known and defined; of these the most important is **Raffinose**, $C_{18}H_{32}O_{16}$, a trisaccharide which is found abundantly in many plant-tissues and products, particularly molasses, eucalyptus-manna, wheat, barley, fungi, bacteria and yeast. It may be distinguished from cane-sugar by its greater solubility in methyl alcohol, and by the fact that it is split by emulsin, yielding d-fructose and melibiose, while cane-sugar is not attacked by this ferment. Hydrolysis by acids yields first d-fructose and melibiose, then the melibiose is hydrolyzed more slowly, yielding d-galactose and d-glucose. Raffinose does not reduce Fehling's solution.

Raffinose is not split by animal tissue-extracts nor by any of the digestive juices with the exception of gastric juice which slowly inverts it owing simply to the fact of its acidity and not to any ferment contained in the juice. As the gastric contents are only distinctly acid for a brief period during digestion we may infer that this mode of splitting raffinose is of no nutritive significance since it must be of very trivial extent. A portion of the raffinose contained in the food is probably absorbed unaltered and excreted as such in the urine, the remainder with the exception of the very small proportion inverted in the stomach, remains unaltered until it reaches the large intestine (cecum) where it is rapidly inverted by the bacteria which inhabit this portion of the alimentary canal and is thus rendered available for nutritive purposes.

We here meet with a phenomenon which is yearly growing of greater significance in our eyes, namely the **Symbiotic Relationship** between the mammals and the bacterial parasites which inhabit their intestines. While the bacterial flora of the intestines constitute a parasitic growth, yet their tenure of the intestine is not wholly to the disadvantage of the host, and through the multifarious enzymes which they produce these organisms render available to mammals foodstuffs which would otherwise be indigestible and excreted unaltered. It is probably for this reason that chickens and rats fed upon a strictly aseptic diet do not grow normally. While in the instance chosen, that of raffinose, the products thus rendered available may not be of indispensible importance to the animal economy, yet in many cases, as for example in the splitting of chlorophyll by the intestinal bacteria, the products which

result (containing methyl-pyrrole groupings), may very possibly be unobtainable by mammals in any other way.

The higher polysaccharides are very imperfectly defined. We have no reliable methods which are available for determining their molecular weights, and we do not know, therefore, how many molecules of sugar take part in their formation. The group is a very large one, and the general formula $(C_6H_{10}O_5)_n$ may be ascribed to the majority of its best-known members, indicating that they are formed by the union of an indefinite number, n, of hexose anhydrides. The following substances are important and typical members of the group: **Starch, Glycogen, Dextrins, Inulin, Pectin, Humin, Cellulose, Gums,** and **Vegetable Mucilages.** It is very important to recollect, however, that these are merely arbitrary terms used to describe very ill-defined members of the series. Thus we cannot be certain that there is only one chemical individual **Starch**; on the contrary, it appears probable that there may be many starches, and starch is certainly known in two widely different forms, to wit: a form insoluble in water and a form which is soluble in water. On the other hand it should be recollected that the differences which are observed between these forms of starch may possibly be purely physical, and not chemical differences at all. We here encounter, in fact, a problem which is presented generally by the colloids, and which we shall meet with again in connection with the proteins.

Starch, inulin, gums, mucilages and glycogen do not reduce metallic oxides in alkaline solutions. They do not, therefore, contain potentially active aldehyde-groups. Dextrins, on the contrary, do contain aldehyde-groups, for they reduce Fehling's solution. With the possible exceptions of glycogen and inulin, the polysaccharides do not form crystals, or at least, they have not as yet been prepared in crystalline form. Water dissolves some of them, others only swell in cold water and dissolve in hot water, others are unaffected by water. Solutions of the polysaccharides do not taste sweet unless held in the mouth for a sufficient period to enable the diastase (**Ptyalin**) in the saliva to bring about hydrolysis. Solutions of the polysaccharides are optically active. The higher polysaccharides do not diffuse through parchment paper, thus behaving typically as colloids. They do not form compounds with phenylhydrazine.

The polysaccharides play a wide variety of parts in the vegetable kingdom. In the first place, they serve as reserve materials, or stores of sugar, laid up against a future time of need; such a part is that played by starch (or vegetable glycogen, as it may be called in analogy to animal glycogen, which plays a similar part in the animal economy) and also by inulin. The gums and mucilages, on the contrary, serve, in part at least, to close up injuries and protect them while healing. The celluloses, again, have yet another function to perform. They, or their derivatives, constitute the supporting tissues of plants, just as bones or exoskeletons constitute the supporting tissues of animals.

The **Celluloses** (the plural is employed because it appears highly

probable that there are many modifications of cellulose), are insoluble in all ordinary solvents, such as water, alcohol, ether, and so forth. Cellulose dissolves, however, in solutions of many metallic salts in the presence of excess of strong acid, for example in zinc chloride in acid solution, and in the hydrochloric acid solutions of antimony, mercuric, or bismuth chlorides. The requisite condition for solution appears to be, the presence of a salt of a weak metallic base in acid solution. Another solvent for cellulose is an ammoniacal solution of cupric oxide, known as "**Schweitzers' Reagent.**" In the presence of concentrated sulphuric acid, sulphuric-acid esters of cellulose are formed and pass into solution. If this solution be diluted and boiled, glucose is formed and glucose only, hence cellulose is an anhydride of glucose. A preliminary stage in this hydrolysis is the formation of **Amyloid**, a soluble colloidal substance which resembles starch in yielding a blue color with iodine.

Cellulose is indigestible by any of the ferments contained in or produced by mammalian tissues. It is, however, digestible by bacteria, and as much as seventy per cent. of unlignified cellulose may be dissolved *in vitro* by the juices from the lower intestine of the horse. The products of this form of digestion are not sugars, but carbon dioxide, methane and fatty acids. Human beings have been found to utilize as much as forty per cent. of young and tender cellulose, doubtless through the agency of the intestinal bacteria. Hence the nutritive value of cellulose, especially in animals such as the cow and horse which possess very long intestines, is by no means negligible. But the celluloses are of significance to the animal economy from yet another point of view. By virtue of their incomplete digestibility they communicate bulk and substance to the feces and thus facilitate their passage through the intestines, in the first place by bringing about a favorable distention of the intestinal muscular walls, and in the second place by furnishing these muscles with material upon which to exert leverage. Prior to the introduction of "War-breads" the tendency of our times was to eliminate indigestible carbohydrates more and more thoroughly from the diet and the prevalence of intestinal stasis and chronic constipation in modern communities is doubtless attributable, in part at least, to this "refinement" of our foodstuffs. A crude endeavor to correct this deficiency in our diet is frequently made by mixing bran or other coarsely ground cellulose-rich materials with the flour from which bread is made. This remedy may in many instances, however, be worse than the disease, for the ingestion of large, horny and sharp-edged indigestible fragments with the food may lead to lacerations of the intestine, and consequent inflammatory reactions or enteritis. What is required is finely ground cellulose-rich material, such as our ancestors enjoyed when they ground up their grains by hand between two hard stones. Agar is frequently employed to communicate indigestible bulk to the diet or, in recent years, heavy tasteless petroleum oils, but in administering these substances we are merely

striving to remedy the consequences of a totally unnecessary dietary habit which arises from a threefold origin of public ignorance, a fancied superiority of things which are white, and therefore "pure," white bread, white eggs, white (*i. e.*, sulphured) dried fruits, white sugar (made to appear white by the addition of litmus) and so forth, and in the irresponsible self-interest of millers and bakers. The War, through the introduction of more thorough utilization of grains to make "War flour" and "War bread" has thus no doubt proved a veritable blessing in disguise to many chronic sufferers and it is not improbable that the reinstatement of our former foolish and wasteful habits of milling will be prevented or delayed by a more general public recognition of the beneficial rôle of indigestible residues in the food.

Cellulose occurs almost exclusively in the vegetable kingdom. It is found, however, in the shells of *Tunicata*. Otherwise it is unknown in the animal kingdom. In the cell-walls of plants, not only true cellulose is found but other cellulose-like substances, some of which yield not only glucose, but other sugars, even pentoses such as arabinose, xylose, etc. Polysaccharides which yield only pentoses on hydrolysis are also found, and are known as **Pentosans.**

As the cell-walls of plants advance in age they undergo a peculiar change resulting in the acquirement of greater rigidity. This process is known as **Lignification.** The exact nature of the change which occurs is not known, but it has been suggested that **Lignin** is formed from cellulose by the formation of compounds with aromatic derivatives.

The vegetable gums and mucilages are a very heterogeneous group of polysaccharides. The gums are insoluble, the mucilages soluble in water. The majority of them yield galactose and arabinose when hydrolyzed by dilute acids. **Agar-agar,** so widely used in culture-media for bacteria, is a representative of this class of carbohydrates; it is derived from certain marine algæ. From marine algæ of the *Fucus* type is also obtained a polysaccharide yielding pentoses on hydrolysis which is designated **Algin.** It is a colloidal substance which behaves like a weak acid, forms insoluble salts of aluminium and lime, and is employed as a waterproofing material and a substitute for size.

Closely related to the gums and mucilages is a group of substances, the **Pectins,** which are of very great industrial importance inasmuch as they are responsible for the gelation of fruit-jellies. The pectins are white amorphous gelatinous substances, which form colloidal solutions in water, do not reduce Fehling's solution, and yield galactose, glucose and pentoses on hydrolysis by acids. They are believed to be derived by partial hydrolysis, due to the organic acids present in fruit-extracts, from a series of parent-substances, the **Pectoses,** which are present in plant-tissues in the form of insoluble calcium salts. The pectins are converted by dilute alkalies or by the ferment **Pectase** into **Pectic Acid,** the calcium salt of which is insoluble in water and forms jellies. Since pectase is destroyed by heat, the formation of fruit-jellies by extracting fruits with hot sugar-solutions is not to be

attributed to the action of pectase, but rather to the production of insoluble jelly-forming substances from pectose or pectin by the hydrolyzing action of the fruit-acids. The pectins are not hydrolyzed by diastases, they are, however, hydrolyzed by special enzymes, the **Pectinases**, found in malt and in certain moulds which liquify pectin jellies with the production of reducing sugars.

Coming now to those polysaccharides which are primarily of nutritive importance, **Starch** is the form in which sugar is chiefly stored up by plants for future consumption, although cane-sugar, inulin and other carbohydrates frequently play a similar part.

Starch is found in the greatest amounts in those portions of plants which are subsequently to be drawn upon for the materials of growth. Thus seeds, roots, bulbs, tubers and the pith of deciduous trees in winter are particularly rich in starch, this carbohydrate frequently comprising as much as eighty per cent. of the dry weight of the material. The starch is stored up in these tissues in the form of stratified granules, which differ characteristically in form and size in different plants. It is by means of these characteristics of form, size and stratification of the granules that we can tell very readily whether a starch alleged to have been derived from one specified source has or has not been adulterated in the pursuance of "legitimate business enterprise" with starch derived from some other and cheaper source.

The concentric rings, or stratifications of starch-grains represent their gradual growth, and intimate that the growth of starch-grains takes place rhythmically, periods of desposition alternating with periods of rest. Starch is only slightly and very slowly changed by cold water, but in hot water the grains swell up and finally burst, forming what is known as "starch-paste." Neither starch nor starch-paste reduces metallic oxides in alkaline solution.

A very familiar test for starch is the formation of a very deep indigo-blue coloration when it is acted upon by iodine solutions in the presence of hydriodic acid or of an iodide. The color disappears on boiling and reappears on cooling. In applying this test it is necessary to remember that it is not given in the presence of excess of reagents which are oxidized by iodine, such reagents, for example, as hydroxides of the alkalies, or sulphurous or arsenous acids. It is in connection with this test that we meet with very clear indications that starch is not a homogeneous chemical unit, for varieties of starch are known which do not give a blue, but a reddish-brown or a "port-wine" color with iodine. We do not know to what these colorations are due, or whether they are specific, *i. e.*, yielded by one chemical individual alone, or generic, *i. e.*, yielded by a group of similar chemical individuals.

On boiling starch with dilute mineral acids, glucose and only glucose is obtained. Starch is therefore an anhydride of glucose. If the acid is allowed only to act upon the starch in the cold, or with very gentle heating, a modification of starch, known as "soluble starch" is obtained. If we act upon starch for several weeks with cold dilute mineral acids,

or for an hour with four per cent. sulphuric acid at 80° C., we obtain "**Amylodextrin,**" which yields a port-wine coloration with iodine. Further hydrolysis of the amylodextrin yields a mixture of simple dextrins which give no color with iodine ("**Achroödextrin**"); still further hydrolysis yields **Glucose**, an intermediate product of hydrolysis being **Maltose** which, however, in the acid-hydrolysis of starch, is immediately broken down into glucose, so that in the hydrolysis of starch by acids maltose is only transiently present in the system. In the hydrolysis of starch by diastatic ferments, however, unless **Maltase** be also present, the final product of hydrolysis is the disaccharide maltose, the intermediate stage of hydrolysis being so far as we know, similar to those observed in the hydrolysis of starch by acids. The hydrolysis of starch takes place, therefore, step by step, with the production of intermediate stages of hydrolysis before the final product, glucose, is obtained. We shall meet with analogous phenomena among the proteins, and if we draw a parallel, which is of course only justifiable in a formal, not in a chemical sense, between the hydrolysis of starch and the hydrolysis of proteins, then we would have the following table of analogues:

Starch	analogous to	Proteins
"Soluble starch"	" "	Albumoses
Amylodextrin	" "	Peptones
Achroödextrins	" "	Polypeptids
Maltose	" "	Dipeptids
Glucose	" "	Amino-acids

Inulin, a polysaccharide found in the tubers of dahlias, and in other situations, bears the same relationship to fructose that starch does to glucose. On hydrolysis by acids it yields only fructose; it is not hydrolyzed by any of the diastatic ferments which hydrolyze starch or glycogen.—It is, however, hydrolyzed by a special ferment **Inulinase.** Inulin differs very markedly from starch, in that it dissolves readily in warm water with the formation of a solution instead of a paste, and it yields a yellow color with iodine.

In various other situations in the vegetable kingdom other polysaccharides resembling starch and inulin are found, differing from these, however, in certain characteristics. Thus we have **Amylin, Lavosin, Cerosin,** and **Secalin,** etc., found in grain-seeds, some of which yield glucose on hydrolysis, others fructose. In *Lupinus luteus* is found **Galactin,** a polysaccharide which yields only galactose on hydrolysis. In *Lichens* is found a polysaccharide, **Lichenin,** which yields only glucose on hydrolysis by acid, but which, curiously enough, is not hydrolyzed by diastatic ferments. It yields a yellow color on treatment with iodine.

Glycogen is to the animal economy what starch is to that of the plant. It was observed by the distinguished French investigator, Claude Bernard, in 1848, that the sugar-content of the liver, excepting after starvation, is very high. He further found that the sugar which the

liver yields on standing is not present as such, but in a form resembling starch, which is rapidly hydrolyzed by enzymes contained in the tissues, or by acids, yielding glucose. Glycogen may be prepared from fresh liver by extracting the tissues with strong potassium hydroxide solution, which decomposes the proteins but does not hydrolyze the glycogen, and then precipitating with alcohol. If the liver be allowed to stand before extraction, a much smaller quantity of glycogen will be obtained, and simultaneously it will be found that sugar has appeared in the liver. If the liver be heated to boiling before being allowed to stand, the glycogen does not disappear and no increase in the sugar content of the liver is observed. Evidently, therefore the disappearance of glycogen in the liver on standing is due to the action of a hydrolyzing ferment which is destroyed or inactivated by heating.

Glycogen, although like starch, an anhydride of glucose is nevertheless readily and sharply distinguishable from starch. It forms when pure a fine white amorphous powder. Its molecular weight is unknown. It dissolves in cold water, forming opalescent solutions, but it is a typical colloid and does not diffuse through parchment. With iodine glycogen yields a reddish-brown or port-wine coloration which disappears on heating and reappears on cooling.

The hydrolysis of glycogen, like that of starch, takes place in step-like stages. Intermediate products of hydrolysis are dextrins and maltose. In the absence of maltase the diastatic ferments hydrolyze it as far as the maltose-stage and then their action stops. It is not by any means certain that there is only one glycogen or that there are not a variety of different reserve-carbohydrates in animal tissues, but if this is the case no means has yet been found of positively separating and identifying them.

Glycogen is found in a variety of tissues, but the chief storehouses in the vertebrates are the liver and the muscles. In invertebrata glycogen occurs in organs which correspond in function to the liver. It also occurs in the protoplasm of unicellular animals and is abundant in yeast. It appears never to occur in the nucleus.

The glycogen which is stored up in the striated and smooth muscles of the vertebrata is of peculiar significance, in that it stands quantitatively in direct relation to the work which the muscles perform. As the muscles do work, glycogen disappears from them. As might be expected, therefore, the percentage of glycogen in muscle varies very much in different animals and under different conditions. The following figures, given by Cramer, show this very clearly:

Animal.	Muscle.	Glycogen, per cent.
Dog Number 1	Biceps brachii	0.17
	Quadriceps femoris	0.53
Dog Number 2	Biceps brachii	0.25
	Quadriceps femoris	0.32
Dog Number 3	Dorsal musculature	0.135
	Posterior adductors	0.077
Dog Number 4	Dorsal musculature	0.417
	Posterior adductors	0.444

Glycogen is also found in glandular, epithelial and connective tissues and in the brain. The distribution of glycogen in the body is very variable; the following figures were obtained by Schondorff, employing dogs which had been well fed with carbohydrates and meat shortly before death:

One hundred grammes of glycogen were distributed in different parts of the body in the following proportion in seven dogs employed:

	Minimum observed.	Minimum observed.	Average.
Blood	0.04	0.001	0.015
Liver	56.74	20.09	37.97
Muscle	62.55	31.22	44.23
Bone	12.88	5.36	9.25
Skin	11.38	1.42	4.49
Viscera	7.30	0.38	3.81
Heart	0.28	0.08	0.17
Brain	0.23	0.04	0.09

It will be observed that the heart-muscle, which is in continual activity, contains very little reserve-stock of carbohydrates. It is evidently unable to accumulate a reserve or capital of carbohydrate and maintains its activity upon its current income. With this may be correlated the fact that after each beat of the heart a definite and relatively lengthy period occurs, the "refractory period" during which even the application of stimuli fails to elicit a contraction from the heart-muscle, whereas ordinary striated muscle, containing abundant stores of reserve carbohydrate, may be stimulated repeatedly at exceedingly brief intervals until relaxation between the contractions becomes a mechanical impossibility, and the contractions fuse into one "tetanic" contraction which relaxes only when the muscle becomes exhausted and its stores of glycogen depleted.

It will be noted, also, that the percentage of glycogen in the blood is extraordinarily low. In fact it appears that the only form in which carbohydrate material circulates in the Vertebrata is that of glucose, and that this is also the only form in which carbohydrate food is utilized by the tissues for the production of energy, or the manufacture of reserve-materials. Now the carbohydrates of the food are usually ingested in the form of starch, glycogen, and other polysaccharides, or in the form of disaccharides, such as cane-sugar or lactose, and these carbohydrates are readily utilized by the organism. Preparatory to utilization therefore, these carbohydrates must undergo elaborations and transformations resulting in the formation of glucose.

AMINO-POLYSACCHARIDES.

The hydrolysis of proteins which contain a glucosamin radical yields in some instances an amino-disaccharide, presumably diglucosamin. The most important amino-polysaccharide in the animal economy is, however, **Chitin**, which forms the exoskeleton of the *Insecta* and the

Crustacea. It may be obtained in colorless semi-transparent lamellæ which are stained reddish-brown by iodine; on addition of sulphuric acid or zinc chloride the color changes to blue or violet. Hydrolysis with strong acids yields about seventy-five per cent. of d-glucosamin. Chitin also contains acetyl radicals which are liberated as acetic acid on fusion with alkali.

Prolonged treatment with alkali in the cold leads to the formation of "soluble chitin" which is diffusible through parchment, but has an extraordinary affinity for water, carrying the water in the dialyzer with it as it traverses the parchment, and withholding it from the cavity of the dialyzer against hydrostatic pressure. Other products of the partial hydrolysis of chitin are crystallizable (chitosans).

GLUCOSIDES.

The glucosides are a large and important class of substances, occurring in great variety in certain vegetable tissues, and also in exceedingly important tissues and localities in the animal body. They yield monosaccharides on hydrolysis and other radicals which differ widely in different glucosides.

Reference has already been made to the glucoside **Amygdalin** which occurs in the kernels of cherries and almonds and is hydrolyzed by the ferment **Emulsin**, yielding glucose, hydrocyanic acid and benzaldehyde. Various species of the *Cruciferæ* contain irritant glucosides, notable among which are **Sinigrin** or **Potassium Myronate** in the oil of black mustard, obtained from the seeds of *Sinapis nigra*, and **Sinalbin**, in the oil of white mustard, obtained from the seeds of *Sinapis alba*. Both sinigrin and sinalbin are hydrolyzed in the presence of water by a ferment, **Myrosin**, which occurs in the tissues of the plants from which they are obtained. The products yielded by the two glucosides are, however, very different, sinigrin yielding dextrose, potassium bisulphate, and allyl isosulphocyanate, while sinalbin yields dextrose, sinapin sulphate (a sulphate of an alkaloid) and methyl phenyl isosulphocyanate. Both glucosides are intensely irritant when applied to the skin, and are utilized for this purpose in therapeutics.

Glucosides of great therapeutic importance are also found in the leaves and seeds of *Digitalis purpurea*, *Strophanthus* and *Scilla*, and comprise the most important active constituents of the pharmacopœial preparations made from these plants. They have a characteristic action upon heart-muscle of which advantage is taken in the medical treatment of cardiac affections. The same plants also contain glucosides which are either without effect upon the heart, or else have an effect which is of secondary importance. Some of these glucosides are members of the saponin series and contribute to the effectiveness of aqueous extracts of the plants by holding in solution substances which would otherwise be insoluble in water.

From a biochemical point of view, and in our present state of knowledge, perhaps the most noteworthy glucosides which occur in plant-tissues are the various members of the **Saponin** and **Sapotoxin** group of glucosides These substances are found in a very great variety of plant-tissues, but especially in *Quillaja*, (soapbark), *Saponaria* (soapwort), *Cyclamen* (cyclamin), *Solanum* (nightshade and potato) and *Smilax* (sarsaparilla). These glucosides behave like weak acids and are split on hydrolysis with acids into sugars and other substances which are for the most part, as yet undefined. They possess to a very remarkable degree the property of reducing the surface-tension at surfaces in contact with water in which they are dissolved and coating these surfaces with an insoluble film, with the result that the forces tending to cause coalescence of bubbles are very much reduced, so that the water containing saponins form froths like soap-solutions, when it is shaken up with air. Hence the names "soapbark," "soapwort," etc. For the same reason they have the property of holding otherwise insoluble substances in solution or suspension, since the suspended particles have less tendency than usual to clump together and thus form masses large enough to fall out of the solution.

The saponins and solanins readily dissolve or form colloidal solutions of a variety of fatty substances, particularly the **Lecithins**, an important group of phosphorus-containing fatty substances which will fall under discussion repeatedly in future chapters. They also form, in many cases, insoluble compounds with **Cholesterol**, an aromatic alcohol, which is found associated with lecithins in all living tissues.

The power of the saponins to dissolve fatty substances is undoubtedly the origin of their remarkable action upon red-blood corpuscles, the stroma of erythrocytes being very rich in lecithins and other fatty substances. As little as one part of cyclamin added to 100,000 parts of blood causes complete liquefaction or **Hemolysis** of the stroma of the corpuscles with resultant setting free of the enclosed hemoglobin, while liquefaction of a proportion of the corpuscles is brought about by even smaller amounts. Cholesterol tends to prevent this action of the saponins by combining with them to form insoluble compounds, and hence blood serum or plasma, since it contains a small amount of cholesterol, to some extent inhibits the hemolytic action of the saponins.

A saponin, digitonin, which occurs in *Digitalis* but is devoid of action upon the heart, is employed in the quantitative estimation of cholesterol.

In animal tissues glucosides are found especially among the decomposition-products of **Nucleic Acids** and in the tissues of the brain. The nucleic acids will fall under special consideration in a later chapter and it need merely be stated here, in passing, that they are phosphoric acid compounds of glucosides, the **Nucleosides**, which yield either d-glucose or d-ribose and nitrogenous bases on hydrolysis. A nucleoside is also found in minute traces in the blood and exerts an action upon the egg-cells of the Sea-urchin (*Strongylocentrotus*) similar to that

of a saponin; it is, however, devoid of lytic action upon the red blood-cells themselves.

The glucosides in the brain, the **Cerebrosides**, occur in complex fatty compounds which yield the free glucosides, **Phrenosin** and **Kerasin** on partial hydrolysis. They also exist in part preformed in brain-tissue, or at any rate can be directly extracted therefrom by solvents such as pyridine or hot alcohol containing benzole or chloroform. These substances are not confined to nervous tissue but are also present in small amounts in the kidney and liver and probably in other organs as well. They also occur in the yolks of eggs.

The cerebrosides are nitrogen-containing substances which are hydrolyzed by acids, yielding fatty acids, galactose, and a nitrogenous base, **Sphingosine**, which is a diatomic amino-alcohol containing unsaturated linkages:

$$C_{13}H_{26}CH:CH.CHOH.CHOH.CH_2NH_2$$

The fatty acid which is yielded by **Phrenosin** is a hydroxy-acid, **Cerebronic Acid**, $C_{25}H_{50}O_3$, while **Kerasin** yields **Lignoceric Acid**, $C_{24}H_{48}O_2$. The two cerebrosides differ furthermore in their solubilities, phrenosin being almost insoluble in boiling acetone, while kerasin is readily soluble. Both cerebrosides are insoluble in water or in ether, but they dissolve in hot alcohol, from which they crystallize in needles or plates on cooling. Solutions of phrenosin are dextrorotatory, those of kerasin being levorotatory.

With sulphuric acids the cerebrosides yield, first a yellow, and later a purple-red coloration. In the presence of cane-sugar and sulphuric acid, they yield a purple coloration immediately. This reaction is attributable to the sphingosine radical.

Cerebrosides are absent in the brains of fetal animals, but with the advance of medullation they appear in abundance. It is therefore assumed that the cerebrosides are constituents originating in medullary sheaths rather than in the axons or nerve-cells.

THE CARBOHYDRATE ESTERS.

Phosphoric acid esters of d-glucose and d-ribose occur among the products of the partial hydrolysis of nucleic acids. They will fall under more extended consideration in a later chapter.

Sulphuric acid esters, the **Glucothionic Acids** have been found by Levene and Mandel in a variety of animal tissues, the nature of the carbohydrate radical is not yet established.

A sulphuric acid ester of an amino-polysaccharide, **Chondroitin-Sulphuric Acid**, $C_{18}H_{26}NO_{13}HSO_4$ occurs in important amounts in bones and other sclerous tissues and also in the walls of the great arteries and in certain pathological tissues. It is a normal constituent of urine in very small amounts. It is soluble in water, yielding levorotatory

solutions, and is precipitable from aqueous solutions by alcohol. Hydrolysis by dilute hydrochloric acid yields sulphuric acid and **Chondroitin**:

$$C_{13}H_{26}NO_{13}HSO_4 + H_2O = C_{13}H_{27}NO_{14} + H_2SO_4$$

chondroitin sulphuric acid + water = chondroitin + sulphuric acid.

Chondroitin reduces Fehling's solution. On further hydrolysis it yields d-galactose and d-glucuronic acid; it appears to be a compound of glucuronic acid and an amino-hexose, **Chondrosamin**, or amino-galactose.

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CHAPTER V.

THE HYDROAROMATIC DERIVATIVES: THE CYCLOSES, CHOLESTEROL AND CHOLIC ACID.

GENERAL CHARACTERISTICS.

A class of bodies here claims our consideration, the members of which, while chemically distinct, are, in their physical behavior and physiological properties intermediate in character between the carbohydrates and the fats. At the one extremity we have the cycloses, which although polyatomic alcohols, nevertheless resemble sugars in their solubility in water, their percentage-composition which is represented by the formula $C_6H_{12}O_6$, and their decidedly sweet taste. At the other we have cholesterol and the cholesterol esters or waxes which resemble the fats in their insolubility in water and solubility in organic solvents, and which are constantly associated with fats and fatty substances in the tissues in which they occur. They all contain a reduced benzole-ring and are thus related to the **Terpenes**; they are furthermore hydroxy-derivatives and thus yield a variety of color-reactions which depend upon the presence of a hydroxyl radical in the benzole-ring.

The extreme importance of these substances in the life of tissues has only very recently come to be suspected, but the variety of parts they are now known to play in essential activities of the living cell is so extensive that we have come to regard them as constituting a very significant factor indeed in the life-economy. Thus **Inosite** in combination with phosphoric acid is an important constituent of seeds and the rapidly growing parts of plants, while in animal tissues inosite is found in a variety of situations and forms an integral part of the molecule of the active principle of the anterior lobe of the **Pituitary Gland.** Cholesterol is found wherever fats occur in animal tissues, and the remarkable effects which it exercises upon the growth of epithelial tissues,[1] show that it plays an important physiological rôle. Cholesterol esters or **Waxes** occur in abundance in vegetable tissues, while in mammals they occur in noteworthy amounts in the fatty sheaths of medullated nerves, and in the cortex of the **Suprarenal Gland. Cholic Acid**, which is probably a derivative of cholesterol, occurs combined with amino-acids (amino-acetic acid or ethyl amino-sulphonic acid) in the bile, and the salts which these acids form with sodium, play an essential part in accomplishing the digestion and assimilation of fats.

[1] Cf. Chapter xx.

It is questionable whether animal tissues are able to accomplish the synthesis of any of these substances; in fact all the evidence at present available contributes to show that they cannot, and that we are absolutely dependent upon vegetable tissues for our supplies of these very essential materials. The investigations of Gardner, Denis Chalatov and Anistchakov have shown that addition of cholesterol to the dietary in abnormal amounts, increases the cholesterol-content of the tissues, while a diet extremely deficient in cholesterol-results in a like deficiency of cholesterol in the blood and tissues. On the other hand, in vegetable tissues terpenes and terpene-derivatives abound so that the ultimate source of cholesterol in the diet would appear to reside in these products of the synthetic activity of plants.

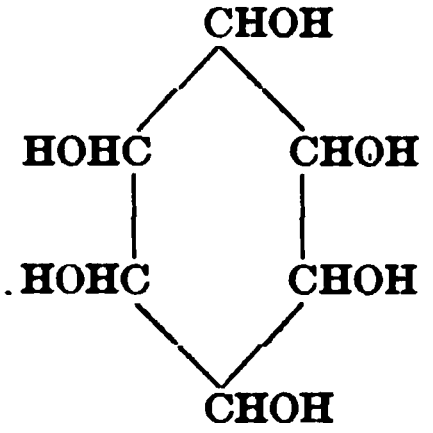
The power of the animal organism to destroy cholesterol is very limited, and if a considerable excess be administered in the diet, the unutilized cholesterol is stored away in various tissues, particularly in the liver, spleen and suprarenal bodies. In certain animals, for example rabbits, but not in others, the excess of cholesterol is in part deposited in the interior of the arterial walls, leading to the formation of lesions, which simulate arteriosclerotic lesions of the arteries in human beings. The normal channel of excretion of cholesterol would appear to be the bile, in which it is present in part in the form of unaltered cholesterol, and in part in the form of cholic acid, combined with amino-acetic acid or amino-ethyl-sulphonic acid to form the "bile-acids." Both of these substances are in part reabsorbed from the intestine, so that there is a tendency for cholesterol and its products to circulate in the body, and accumulate in the tissues. Of course this process cannot go on unchecked, otherwise the accumulations of cholesterol in the tissues would soon extinguish their functional activities. It appears possible from the abundance of cholesterol esters in the suprarenal cortex, particularly during cholesterol overfeeding, that the suprarenal glands may play a part in assisting to eliminate or destroy cholesterol, but regarding the nature of the ultimate products which may be formed in this process we are entirely in the dark. Inosite, on the other hand, which contains within itself a much higher proportion of oxygen than cholesterol, is partially oxidized by animal tissues and the products of its oxidation appear to be indistinguishable from those of carbohydrate-metabolism.

Not even inosite, however, and still less cholesterol are of importance from the purely nutritive aspect, *i. e.*, as sources of energy. The calorific value of the hydro-aromatic fraction of the diet is so small as to be negligible in comparison with the total. Their significance lies elsewhere, and if we revert to the analogy of inanimate machines we must class them with the lubricants and other accessory substances which are essential to the smooth running of the machine, rather than with the fuel which supplies the energy of the machine. Indirectly, indeed, they must contribute to the available energy-value of the diet by permitting its more efficient consumption, just as the judicious

employment of lubricants will diminish the necessary consumption of gasoline in an automobile-engine. Their influence upon the nutrition of animals is indirect, however, and not direct, and the hydro-aromatic derivatives must for this reason be classified as **Accessory Foodstuffs** or foodstuffs which are primarily utilized for other purposes than the production of work and heat, or the building up of the structural elements of tissues.

THE CYCLOSES.

The hydro-aromatic compounds which lie nearest to the carbohydrates in their physical properties and physiological behavior are the **Cycloses**, or hexa-hydroxy-benzoles, which are represented by the formula:



A number of isomeric compounds are represented by this formula, differing from one another in the arrangement of hydrogen and hydroxyl groups about the carbons. The form which occurs in animal tissues is optically inactive, the levo- and dextrorotatory carbons being balanced and equalized within the molecule. This cyclose is designated **Inosite.** In vegetable tissues it is found widely distributed, occasionally in the form of ester-like compounds (dambonite, bornesite), but chiefly in the form of the hexaphosphate, the calcium-magnesium salt of which is known commercially as **Phytin.** This substance occurs particularly abundantly in seeds and grains, the husks of which also contain a ferment, **Phytase**, which is capable of splitting the compound, in aqueous solution, into inosite and phosphoric acid, a hydrolysis which otherwise can only be accomplished completely by exposing the substance in acid solution to a temperature very considerably above that of boiling water. Intermediate steps in the hydrolysis of inosite hexaphosphate by phytase are the tri- and mono-phosphates which do not, however, occur preformed in the tissues of grains.

In mammals i-inosite is found in small amounts in muscular tissue, from which it was first obtained and recognized as a distinct chemical entity. It is also found in combination with a complex fatty substance, containing phosphorus and nitrogen, in the tissue of the anterior lobe of the pituitary body. This compound, to which the name **Tethelin** has been applied, is probably the physiologically active principle of the gland. On somewhat prolonged hydrolysis by alkalies and acids the substance breaks up and yields free i-inosite.

Inosite is readily soluble in water and alcohol and is obtained in the

form of fine white acicular crystals by the addition of ether to an alcoholic solution. It has a sweet taste, but being neither actually nor potentially an aldehyde or ketone, it does not reduce metallic oxides in alkaline solution, and hence, of course, does not reduce Fehling's solution. It is precipitated from aqueous solutions by lead acetate containing an excess of lead oxide ("basic lead acetate").

Inosite may be recognized by the above peculiarities, by its melting-point (225°), and by the following characteristic reactions:

Gallois' Reaction.—A drop of inosite solution is mixed with a drop of mercuric nitrate solution and heat gently applied until the water has evaporated. A yellow color at first appears which changes on further heating to a deep red. This color disappears on cooling, and reappears on reheating.

Scherer's Reaction.—A few crystals of inosite are dissolved in a drop or two of nitric acid of specific gravity 1.2, and an equal volume of ten per cent. calcium chloride solution is added and the same volume of a one per cent. solution of platinic chloride. This mixture is evaporated to dryness and the residue heated, when a rose-red color appears, which disappears on cooling, and reappears with a bluish nuance on reheating.

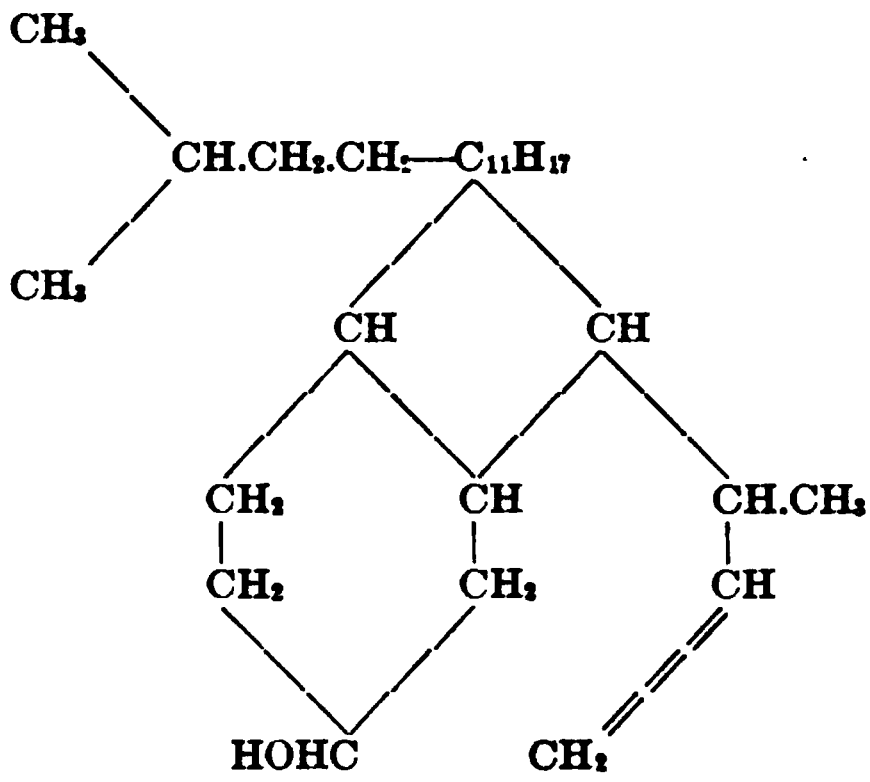
Inosite is found in small amounts in normal urine, and the amount increases in certain pathological conditions, particularly in diabetes insipidus and in Bright's disease. The administration of inosite in unusual amounts by mouth gives rise to transient diarrhœa and to an increase in the **Creatinine** output in the urine, a fact which, in the light of considerations which will be detailed in subsequent chapters, may possibly indicate increased destruction of tissue-substances. Only a very small proportion of the inosite administered by mouth is excreted in the urine, the remainder being oxidized and eliminated in the form of products which are apparently indistinguishable from those of ordinary carbohydrate-combustion. In phloridzinized dogs the excretion of d-glucose in the urine, already a maximum, is increased by administration of inosite, and if the additional output of glucose be added to the inosite which is excreted unchanged in the urine, the sum is approximately equal to the inosite administered. Under these circumstances, therefore, the ring-formation appears to undergo a simple splitting with the partial transformation of inosite, molecule for molecule, into glucose.

Cycloses other than i-inosite occur in vegetable tissues but with one exception have not as yet been identified among the constituents of animal tissues. The exception is **Scyllite** which is found in the tissues of the bony (Teleost) fishes. It gives Scherer's reaction and is optically inactive, but it may be distinguished from i-inosite by its very high melting-point; 380° as contrasted with 225°.

In vegetable tissues occur l-inosite in the form of the methyl ester in quebracho bark, d-l-inosite or racemic inosite (a mixture of the d- and l-varieties) in the leaves of mistletoe, and d-inosite in the rosin and needles of conifers, in senna leaves and in India-rubber.

CHOLESTEROL AND THE PHYTOSTEROLS.

Cholesterol, $C_{27}H_{45}OH$, may be represented so far as our knowledge at present extends, by the formula of von Fürth:



it is found in all animal fats or oils, in small quantities, in bile, blood, milk, yolk of egg, the medullated sheaths of nerve-fibers, the liver, kidneys and suprarenal bodies. It is contained in considerable amount in cod-liver oil. Under pathological conditions it is found to constitute a very large proportion of the most frequently occurring type of gall-stones, the conditions which ordinarily hold cholesterol in solution in bile, being in these cases, it appears, deficient. It occurs also in atheromata of the arteries, in tubercular cysts and in carcinomatous tissue.

When precipitated from alcoholic solution by the addition of water, or when deposited in the body, as in gall-stones, cholesterol forms characteristic crystals with one re-entrant angle, resembling flat rectangular plates with one corner knocked out (Fig. 2). These crystals contain one molecule of water and are white, of a waxy consistency, insoluble in water, soluble in alcohol, ether, benzol, etc., and in fatty oils. When crystallized from anhydrous alcohol-ether mixtures cholesterol forms acicular crystals without any water of crystallization. Cholesterol may be held in solution or suspended in emulsified form in water by the addition of soaps, saponins, bile-salts, or lecithin, and it is by this means that it is held suspended in the bile and other tissue-fluids.

As has been stated above, there is reason to suppose that cholesterol may possibly be decomposed in the suprarenal glands, and a portion is possibly converted into cholic acid in the liver, but for the rest, so far as we know at present, the main channel of excretion for cholesterol is the bile. The cholesterol which thus finds its way into the upper part of the small intestine, along with the cholesterol of the food, is in part reabsorbed and in part retained in the intestine until it is voided

with the feces. This latter portion of the cholesterol becomes subject in the lower intestine to the putrefactive action of bacteria, which results in its reduction to a derivative of cholesterol designated **Coprosterol**, containing two additional hydrogen atoms, and represented by the formula $C_{27}H_{47}OH$.

This inefficient method of excretion would lead undoubtedly to a continual accumulation of cholesterol within the tissues, if it were not assisted by some means of destruction of the accumulated excess. The power of the body to destroy cholesterol is, however, very limited, and if cholesterol be administered in the dietary in unusual quantities, it forms deposits in various organs, notably the liver and suprarenal glands, and may ultimately lead to the formation of serious lesions. There is therefore, under ordinary circumstances, rather a delicate balance between the intake of cholesterol in the food on the one hand,

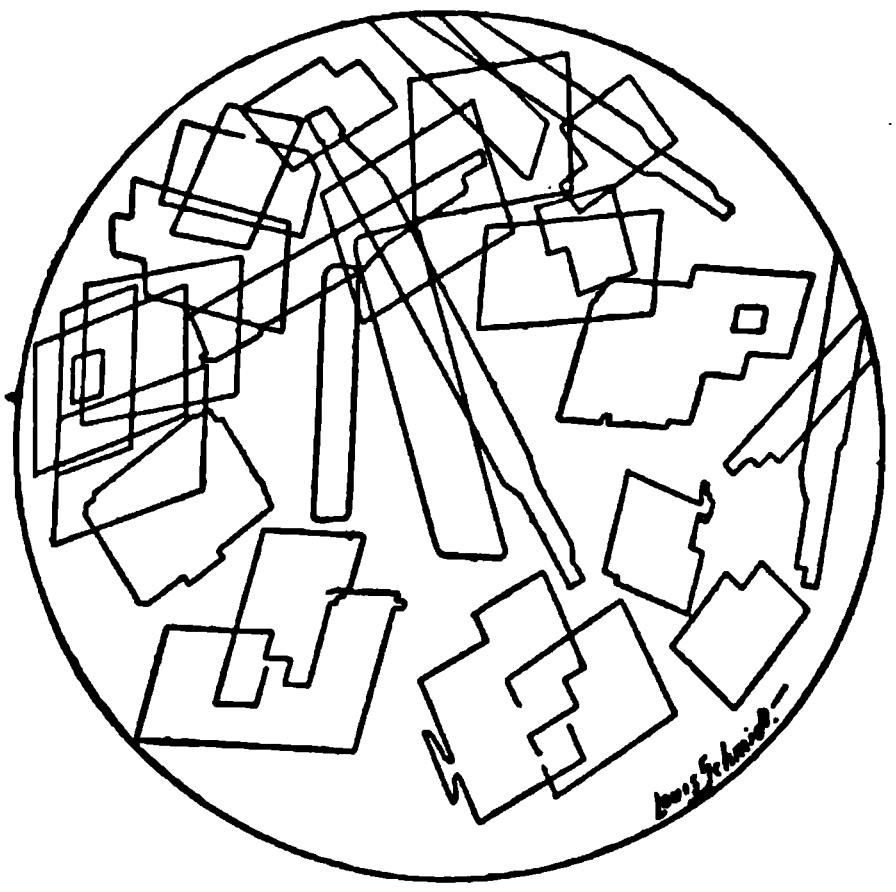


FIG. 2.—Cholesterol crystals. (After Hawk.)

and its output in the feces, and destruction in the tissues on the other. If the power of the tissues to destroy or alter cholesterol is diminished for any reason we may anticipate that the excretory apparatus will be found inadequate, and that cholesterol will accumulate in the body. It is to this that we must probably attribute the accumulation of cholesterol which has been observed by Wacker in the subcutaneous fatty tissues of aged people, the decline in the activity of the tissues which accompanies age probably resulting in a deficient power of destroying cholesterol. It has been observed by Luden that the cholesterol-content of the blood in carcinomatous patients is usually high and that oxidation-products of cholesterol which are present in normal blood are frequently absent in these cases.

The administration of unusual amounts of cholesterol to young animals results in marked effects upon their **Growth**, which will be fully discussed in a later chapter. If cholesterol be administered to animals

(rats) inoculated with carcinomatous tissue, the cancer grows much more rapidly than in normal animals and "metastases" or fresh growths in localities distant from the site of the primary growth, are formed much more numerously and in a much higher proportion of animals. In this connection it is significant to observe that **Carcinoma** is primarily a disease of old age so far as manifest growth or accretion of the parasitic tissue is concerned. It very rarely manifests itself in man before thirty and increases in frequency very decidedly with advancing age, the incidence between the ages of sixty-five and seventy-five being no less than ten times as great as between thirty-five and forty-five. It is, however, impossible to *initiate* carcinomatous growths in animals by administration of cholesterol, unless carcinoma-tissue is already present as a result of inoculation or spontaneous development, so that cholesterol cannot be looked upon as a cause, but rather as a favoring condition of cancer-growth. It must be remembered that our estimate of the age of incidence of carcinoma is founded upon the date at which the growth obtrudes itself upon the attention of the patient or physician. For how long prior to this its beginnings have been actually resident in the body, we have no means of estimating, but judging by the analogy afforded by other growth-phenomena (cf. Chapter XX) we may infer that the date of *origin* of the growth probably precedes by a considerable interval the date of its obvious manifestation, so that despite the fact that cholesterol cannot initiate cancer, the date of its diagnosis, and therefore its "apparent" or "statistical" date of incidence may very possibly be determined by the acceleration of its growth due to an accumulation of cholesterol in the tissues.

Cholesterol yields the following series of color reactions together with others, for description of which the student is referred to special monographs:

Salkowski's Reaction.—Cholesterol is dissolved in chloroform and an equal volume of concentrated sulphuric acid is added. The solution is colored blood-red which changes gradually to purple. If the mixture is poured out in a shallow layer and exposed to the air, the purple changes to blue, then green and ultimately yellow.

Liebermann-Burchard Reaction.—Cholesterol is dissolved in a small amount of chloroform in a dry test-tube, a few drops of acetic anhydride are added and then concentrated sulphuric acid is added drop by drop. The mixture becomes red, then blue and finally, if not too much cholesterol and sulphuric acid have been added, a permanent green.

Obermüller's Reaction.—Dry cholesterol is heated in a glass tube with two or three drops of propionic anhydride until it melts. On cooling the mass turns first violet, then blue, green, orange, and finally red.

Schiff's Reaction.—To dry cholesterol in an evaporating dish add a trace of ferric chloride, strong hydrochloric acid and chloroform, and evaporate the mixture nearly to dryness, when the edge of the residue begins to turn violet. Then add more chloroform, evaporate to dryness and heat. The whole mass turns violet first with a reddish and later with a bluish nuance, and finally a dirty green.

Neuberg-Rauchwerger's Reaction.—This reaction is of exceptional interest because it is also given by the bile-acids and certain other derivatives of the terpenes. A common origin of the bile-acids (cholic. acid) and cholesterol is thus indicated. With rhamnose or better still, with d-methyl-furfurol and concentrated sulphuric acid, an alcoholic solution of cholesterol gives a pink ring, or after mixing the two liquids and cooling, a pink solution.

Lifschütz's Reaction.—Dissolve a few milligrammes of cholesterol in two c.c. of glacial acetic acid, add a few drops of benzoyl superoxide, and boil. On adding four drops of concentrated sulphuric acid to the solution a green coloration is obtained, which rapidly changes to violet, then to blue. Oxidation-products of cholesterol yield this reaction without preliminary treatment with benzoyl superoxide, and in this way oxidation-products of cholesterol have been detected in the blood and tissues, and especially in cholesterol-concretions (gall-stones) in the gall-bladder.

In plant-tissues there are found a variety of substances, the **Phytosterols**, which are more or less closely allied to cholesterol. The best, known of these is **Sitosterol**, an isomer of cholesterol, which occurs in wheat, rye, linseed-oil and the calabar bean. It differs from cholesterol in crystalline form, melting-point (137° contrasted with 148.5° for cholesterol) and optical rotatory power. Its solubilities in various organic solvents, and the color reactions which it yields are similar to those of cholesterol. It is absorbed together with cholesterol from the intestine.

In fungi a series of phytosterols are found which contain a smaller proportion of hydrogen than cholesterol, and furthermore, differ from cholesterol in not yielding Salkowski's reaction.

BILE-CONCRETIONS; AMBERGRIS.

The concretions which occasionally form in the gall-bladder are of three types, formed respectively of **Calcium Carbonate, Bile-pigments** and **Cholesterol.** Each of these types of gall-stones is usually contaminated with a larger or smaller proportion of the constituents of the other types. The cholesterol-stones have a waxy glistening and crystalline fracture, and are frequently deposited in concentric layers. They are often facetted by the pressure of adjacent stones, while their color is sometimes white, but more frequently tinged with bile-pigments.

The cholesterol-stones are the type which most frequently occur in man. The conditions leading to their formation are unknown but it is perhaps a significant fact, in view of the accumulation of cholesterol in the tissues with advancing age, that the incidence of cholelithiasis increases progressively with the advance of years, over 75 per cent. of cases occurring in persons over forty years of age. It is furthermore stated that cholelithiasis is more frequent in carcinomatous than in non-carcinomatous subjects.

On the other hand the deposition of cholesterol may frequently originate, not so much in the abundance of this substance in the bile, as in its diminished solubility therein. An increase in the albumin-content of bile, as in inflammatory conditions, or by the addition of egg-albumin to bile *in vitro* may lead to the deposition of cholesterol and it is stated that certain bacteria, particularly the typhoid bacillus, diminish the solubility of cholesterol in bile which they inhabit.

The proportion of cholesterol in cholesterol-stones varies from sixty-four to ninety-eight per cent. In addition there occur derivatives of cholesterol which yield Lifschütz's reaction without preliminary oxidation, and are probably, therefore, derivatives originating from cholesterol by oxidation. Similar substances are found in the blood of normal persons, but are deficient in or absent from the blood of persons afflicted with carcinoma (Luden).

The biliary concretions of the sperm whale (*Physeter macrocephalus*) are occasionally found floating upon the sea, or cast up upon the shores of oceans inhabited by these mammals. They are found in dull gray or black masses, having a peculiar sweet earthy odor, and form the **Ambergris** of commerce. When taken directly from the intestinal canal of whales it is of a deep gray color, soft consistence and disagreeable odor, but on exposure to air, it hardens and acquires the characteristic odor just described. Ambergris formerly enjoyed a high reputation as a therapeutic agent but its therapeutic virtues probably resided in its scarcity and expensiveness. At the present time ambergris is of importance solely in the manufacture of perfume in which its utility depends upon the rather extraordinary property it possesses, when added to perfumes in minute amounts, of very markedly enhancing their "floral" fragrance.

Ambergris consists in the main, frequently to the extent of eighty-five per cent., of a substance, **Ambrine**, which very closely resembles cholesterol in its solubilities, general appearance and composition. It is insoluble in water, highly soluble in alcohol, ether and oils, and crystallizes in white shining plates.

CHOLESTEROL ESTERS.

Cholesterol esters of the fatty acids are very widely distributed in the vegetable kingdom. In the animal kingdom they are found in the blood and lymph, in the medullated sheaths of nerves, in the cortical tissues of the suprarenal gland and in the secretions of the sebaceous glands. The so-called fat or grease of sheeps' wool, which, when refined is commercially known as "**Lanoline**," consists almost entirely of a mixture of the palmitate, oleate and stearate of cholesterol together with a variable proportion of water.

The fatty acid esters of cholesterol resemble the true fats, or fatty acid esters of glycerol, in their solubility in organic solvents, and insolubility in water. They differ, however, from the fats in the

comparative difficulty with which they are hydrolyzed or "saponified" by alkalies, in their resistance to the action of bacteria, so that they do not become "rancid," and in the property they possess of absorbing or mechanically imbibing a large proportion of water to form a mass which still retains a fatty consistency. For this reason lanoline has of late come to be employed very widely in therapeutics as a vehicle for aqueous solutions of drugs which, through this agency, may be applied as salves.

The cholesterol esters differ from cholesterol is not being emulsifiable in water containing soaps. Acetyl cholesterol $C_{27}H_{45}.OOC.CH_3$ is also devoid of the characteristic action of cholesterol upon the growth of carcinomata. It would seem unlikely that this is due merely to the replacement of a hydroxyl-group by an acetyl-group, more especially since a variety of soluble and insoluble hydroxyl-derivatives of hydroaromatic substances have been found to be devoid of action upon the growth of carcinoma. It appears more likely that the loss of emulsifiability consequent upon the replacement of the hydroxyl-group prevents the distribution of acetyl cholesterol by the blood and tissue-fluids to the cells of the carcinomatous tissue.

The cholesterol esters are of exceptional interest to the physical chemist because they are the substances which were first observed by Lehmann to display the remarkable phenomenon of "fluid crystals" or drops which, while spherical and retaining the characteristics of fluids, nevertheless display evidence, afforded by the unequal refraction of light in different axes, of the possession of an internal crystalline structure. While other bodies are now known which display this peculiarity, the cholesterol esters still constitute a group which is preeminently suitable for the investigation of fluid crystals. They may be very readily obtained by gently heating the esters on a microscope slide until somewhat above the melting-point, and allowing to cool to a little above the melting-point.

According to Adami fluid crystals, presumably containing cholesterol esters, may be observed in the myelin droplets which form during the degeneration of the fatty sheaths of medullated nerves.

THE BILE-SALTS AND CHOLIC ACID.

The mixed bile-salts, sodium glycocholate and sodium taurocholate may readily be obtained from ox-bile by mixing the bile with animal charcoal, evaporating to dryness, extracting with hot alcohol and adding ether to the cooled extract. If the process has been properly performed, a snow-white precipitate of fine acicular crystals ("Plattner's crystallized bile") is obtained which, in one or two crystallizations, may be almost freed from contamination. The two salts may be separated by adding lead acetate to their aqueous solution, by which means the glycocholate is precipitated, while the taurocholate remains in solution. Sodium glycocholate is the most abundant

constituent of the bile-salts in herbivorous animals and in man, but is absent from the bile of carnivorous animals. Sodium taurocholate, on the contrary, only occurs in small amounts in the bile of herbivora and man, while it is abundant in the bile of carnivora.

The bile-salts are readily soluble in water, yielding solutions which, like solutions of the saponins, have a very low surface-tension, foam readily, and hold otherwise insoluble substances in solution or suspension. This is especially true of the lecithins, which are very readily emulsified by bile-salts. The low surface-tension of these solutions is utilized very frequently in manometers for measuring very minute changes of gas-pressure. The solution of bile-salts does not "stick" or form drops on the sides of the containing tube as water frequently does, and the meniscus or surface of the fluid is flatter than that of water, enabling a reading of the height of a column to be made with greater ease and accuracy.

The bile-salts and the free acids are further characterized by the peculiar taste of their solutions, at once bitter and sweet. The dry salts form a very fine light powder which is irritating when it comes into contact with the nasal mucous membranes.

Hydrolysis of glycocholic acid by boiling with barium hydroxide yields **Glycocoll** and **Cholic Acid**:

$$C_{23}H_{39}O_3.CO.HN.CH_2.COOH + H_2O = C_{23}H_{39}O_3.COOH + CH_2NH_2COOH$$

glycocholic acid + water = cholic acid + amino-acetic acid.

while hydrolysis of taurocholic acid yields **Cholic Acid** and amino-ethyl sulphonic acid (**Taurin**):

$$C_{23}H_{39}O_3.CO.HN.CH_2.CH_2SO_2.OH + H_2O = C_{23}H_{39}O_3COOH + H_2N.CH_2.CH_2.SO_2OH$$

taurocholic acid + water = cholic acid + amino-ethyl-sulphonic acid.

The characteristic peculiarities of the bile-acids are determined by their common radical, the cholic-acid fraction. Free cholic acid is almost insoluble in water, but its salts readily dissolve, forming bitter-sweet solutions which are dextrorotatory. The alkali salts of cholic acid, on the other hand, are only sparingly soluble in alcohol, while the free acid dissolves readily in this solvent. Cholic acid may also be recognized by the following characteristic reactions:

Hammarsten's Reaction.—If finely powdered cholic acid be added to a twenty-five per cent. solution of hydrochloric acid, a violet-blue coloration slowly appears which gradually changes through green to yellow.

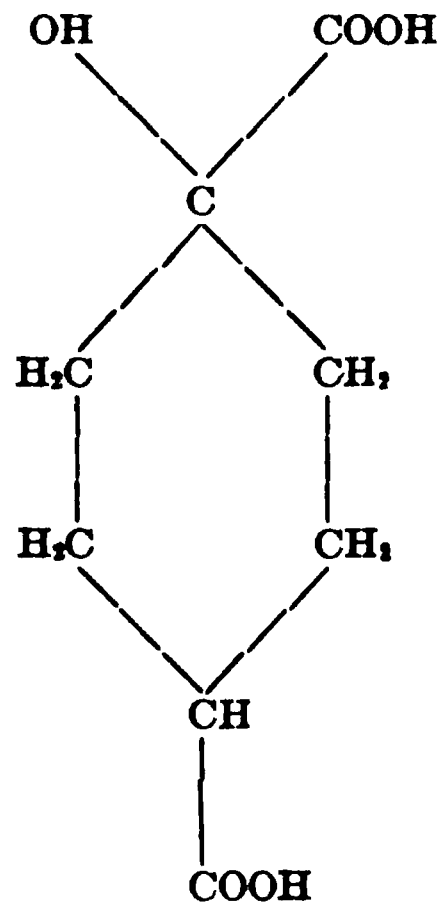
Mylius' Reaction.—If an alcoholic (about five per cent.) solution of cholic acid in alcohol be mixed with two volumes of $\frac{N}{10}$ iodine solution in alcohol, and the mixture slowly diluted with water, microscopic needles of an iodine addition-product are formed which are blue by transmitted light. This reaction is characteristic for cholic acid and is not given by the conjugated bile-acids (glycocholic or taurocholic acids).

Pettenkofer's Reaction.—With a little cane-sugar, on careful addition of strong sulphuric acid, it yields a red coloration. This reaction, which is probably due to furfurol formed from cane-sugar by the action

of the sulphuric acid is not absolutely to be relied upon, since similar reactions (differing from one another, however, in the absorption-spectra of the fluids produced) are yielded by a variety of substances, for example proteins, oleic acid, phospholipins, amyl alcohol and morphine.

Neuberg-Rauchwerger's Reaction.—Also given by cholesterol, which see.

The structure of cholic acid has not yet been fully elucidated, but it appears to be definitely established that it is a derivative of the hydro-aromatic series. The decomposition-products resulting from the variety of procedures contain fractions which are closely related to products which are similarly obtained from other hydro-aromatic derivatives, such as cholesterol, turpentine and camphor, and the identification by Panzer of hydroxy-hexahydro-phthalic acid:



among the oxidation-products of cholic acid leaves very little room for doubt that hydro-aromatic nuclei exist preformed in the undecomposed molecule. Apart from these inferences, however, it is known that cholic acid contains one carboxyl-group and two primary and one secondary alcohol-groups united to a hydrocarbon-complex which contains cyclic linkages. The formula may therefore be written:

$$C_{20}H_{31}\begin{cases}CHOH\\CH_2OH\\CH_2OH\\COOH\end{cases}$$

A related acid which is found in small amounts in ox-bile and also in gall-stones is **Choleic Acid,** which differs from cholic acid in its percentage composition ($C_{24}H_{40}O_4$) and in its relative insolubility in alcohol. It yields a blue compound with iodine and also gives Hammarsten's reaction with hydrochloric acid. **Desoxycholic Acid,** however, which also occurs in bile and in gall-stones yields neither of these reactions, although it is isomeric with choleic acid.

In the bile of animals other than man or the ox are found a variety of acids, which have as yet been very imperfectly studied but which differ in composition and physical characteristics from one another and from cholic acid. A common origin of these substances is probably to be sought in hydro-aromatic radicals contained in the diet and derived ultimately in all probability from vegetable tissues.

The bile-salts are, in part at least, reabsorbed from the intestine, and bile-salts administered by mouth cause a remarkable increase in the secretion of bile, in fact, with the possible exception of salicylic acid, the bile-salts appear to be the only true **Cholagogues** or stimulants of the secretion of bile.[1] When they are injected into the blood or forced into the blood owing to an obstruction of the bile-ducts, leading to icterus or "jaundice," they have a markedly depressant action upon the heart-muscle, slowing the beat very decidedly, and in large amounts they dissolve the red blood-corpuscles just as the saponins do. Under these circumstances bile-salts are probably excreted in part in the urine, but no reliable method of confirming their presence in the urine has yet been devised. For clinical purposes however, this is not an inconvenience since the presence of bile in the circulating blood is always evidenced by the appearance of **Bile-pigments** in the urine which are readily detected in a variety of ways.

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[1] In the opinion of some investigators, however, the increase in the secretion of bile which results from the administration of bile-salts is no greater than that which would be equivalent to the amount of bile-salts administered.

RELATIONSHIP OF CHOLESTEROL TO CARCINOMA:

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CHAPTER VI.

THE FATS.

THE TRUE FATS.

The true fats are compounds, or **Esters** of the fatty acids with the triatomic alcohol glycerol.[1] Thus tripalmitin is formed by the union of three molecules of palmitic acid with one molecule of glycerol and the elimination of a corresponding number of molecules of water.

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + \begin{array}{l} HOOC.C_{15}H_{31} \\ \\ HOOC.C_{15}H_{31} \\ \\ HOOC.C_{15}H_{31} \end{array} = \begin{array}{l} CH_2OOC.C_{15}H_{31} \\ | \\ CH.OOC.C_{15}H_{31} \\ | \\ CH_2OOC.C_{15}H_{31} \end{array} + 3H_2O$$

By the action of alkalies this process is reversed, and the fatty acids which are thus set free combine with the excess of alkali to form soaps. The process of the hydrolysis of fats by alkali is therefore known as **Saponification.**

Monoglycerides, *i. e.*, glycerides containing only one fatty acid molecule, and **Diglycerides** are readily procurable in the laboratory, but they do not usually occur in natural fats unless they have been exposed to the action of fat-splitting enzymes (**Lipases**) or other saponifying agencies. In the **Triglycerides** the fatty-acid radicals need not all be identical and two or even three different fatty acids may be combined with one and the same molecule of glycerol to form neutral fat.

The specific gravity of the fats is less than that of water, and when liquid, or liquefied by heat, those which are insoluble in water float upon the top of it. The fats which are formed from the higher fatty acids are insoluble in water, while the solubility of the lower members in water decreases as the number of carbon atoms in the fatty acid molecule increases. They are soluble in a variety of organic solvents, and form very stable suspensions or emulsions in water in the presence of emulsifying (surface-tension reducing) agents such as soaps, bile-salts, saponins and so forth.

[1] The separation of glycerol from fats was first accomplished by the Swedish chemist Scheele, in 1779. News of this discovery had, however, not yet reached the legislative assembly of one of the allied nations in 1914, with the result that in 1915 a responsible official of the executive, in reply to the inquiry of a legislator stated that it had only recently been discovered that nitroglycerin could be made from fats. It is perhaps time that a civilization which is based on mechanics, physics and chemistry should insist on a rudimentary knowledge of the practical import of these sciences on the part of its legislators and executives.

The fatty acids which are found in the naturally occurring fats belong to two series, the saturated series, represented by the general formula $C_nH_{2n}O_2$ and the unsaturated or oleic acid series represented by the general formula $C_nH_{2n-2}O_2$. In this latter series of acids two of the carbon atoms are united by a double bond or unsaturated linkage which enables them to react very readily with hydrogen, oxygen or the halogens, the double bond being converted into a single one, and the remaining valencies of the carbons saturated by combination with the reacting atoms. The higher fatty acids which occur in nature usually have even values of n and the chain of carbon atoms is not branched.

The lower acids, having small values of n, are formed in the secretions of the sebaceous glands, and in butter, while the tissue-fats and vegetable oils are in the main composed of fats derived from higher fatty acids. Thus in sweat we find:

Formic acid, H.COOH
Acetic acid, CH_3COOH
Propionic acid, C_2H_5COOH
Butyric acid, C_3H_7COOH
Isovalerianic acid, C_4H_9COOH
Caprylic acid, $C_7H_{15}COOH$

These acids are probably secreted in combination with glycerol, but if the sweat be allowed to remain in contact with the skin, the glycerides are attacked by bacteria which hydrolyze them, liberating the free acids, to which the characteristic odor of the "unwashed" is attributable. The odor of the lowest members of the series, **Formic** and **Acetic Acids**, is sharp, reminiscent in the former instance of ants, in the latter of vinegar. **Propionic Acid** has an intermediate odor, while **Butyric Acid** has the odor of rancid butter and **Valerianic Acid** the most intensely disagreeable odor of decomposing perspiration. The highest members of the fatty-acid series are non-volatile and have only very faint odors.

In butter the lowest member of the series is butyric acid, while caproic and caprylic acids also occur together with higher fatty acids particularly **Palmitic** and **Oleic Acids**.

The majority of the tissue-fats are, however, mixtures of the glycerides of **Palmitic Acid**, $C_{15}H_{31}COOH$ and **Stearic Acid** $C_{17}H_{35}COOH$, of the saturated series, with glycerides of **Oleic Acid**, $C_{17}H_{33}COOH$, of the unsaturated series. The separation of the saturated from the unsaturated acids of the higher series may be accomplished by converting them after hydrolysis of the fat, into the lead-salts, and extracting them with ether. The lead-salts of the higher fatty acids of the saturated series are almost insoluble in ether, while those of the unsaturated series readily dissolve in this solvent. The ether extract therefore contains all of the unsaturated fatty acids in combination with lead. The lead may be removed by extraction of the ether with dilute hydrochloric acid, leaving the free fatty acids dissolved in the ether.

THE CHARACTERISTICS OF THE NATURAL FATS.

The various animal fats and vegetable oils differ from one another very strikingly in their physical characteristics and chemical behavior. These differences are in the main determined by the relative proportions in which the glycerides of the three fatty acids above mentioned occur in the fat. The glycerides of oleic acid have the lowest melting-point, those of stearic acid the highest, and hence olive oil, which consists very largely of glycerol trioleate is fluid at ordinary temperatures, while mutton-fat, which contains a high proportion of glycerol tristearate, is solid or semi-solid at ordinary temperatures. The melting-points of the pure fats are as follow:

Triolein	−6.0° C.
Tripalmitin	65.0° C.
Tristearin	71.5° C.

a small admixture of triolein, however, reduces the melting-point of a fat to a very considerable degree.

The chemical reactivity of the fats is also strongly influenced by their content of oleates. The unsaturated bond in oleic acid renders it capable, under appropriate conditions, of directly absorbing hydrogen, being thereby converted into the corresponding saturated acid. The artificial hydrogenation of vegetable oil is now being very largely practised and results in the production of a solid fat, utilizable for a variety of household purposes for which the fluid oil would be unsuited. The significance of the process, however, goes far beyond this. The addition of two atoms of hydrogen to the oleic acid molecule adds considerably to its calorific value, since the heat of combustion of the hydrogen to water is thus rendered available for nutritive purposes. In the aggregate the hydrogenation of vegetable oils adds to the nutritive value of these fats an amount[1] which would otherwise require a very great deal of space and labor to produce. From an economic point of view therefore, and as a means of food conservation, the hydrogenation of vegetable oils is a very desirable thing to encourage. It is true that the vegetable oils fail in important respects to furnish the nutritive equivalent of animal fats, for, as we shall see in later chapters, the animal fats contain accessory foodstuffs which are essential for growth, and even for the maintenance of health, while the vegetable oils are lacking in these. To the extent, however, to which fats are employed in the diet for their mere fuel-value, the vegetable oils are fully equivalent substitutes for the animal fats, and only a small proportion of the total fat-consumption, at any rate in adults, is requisite to furnish the accessory foodstuffs which we acquire from the animal fats. It is probable that there would be no danger of shortage of the accessory foods being caused by the utilization of vege-

[1] Roughly 7 per cent.

table fats, unless meat and dairy products were at the same time very deficient in the dietary.

The unsaturated bonds in the oleates also confer upon them the property of absorbing halogens, and the power of various natural fats and oils to absorb iodine is used as a means of characterizing and identifying them. The **"Iodine Number"** is the number of grammes of iodine which is absorbed by a hundred grammes of fat dissolved in chloroform and treated with a solution of iodine in alcohol or acetic acid. Other characteristics which are employed to differentiate the natural fats are: The **"Hehner Number"** or weight of water-insoluble fatty acids yielded by 100 grammes of fat; the **"Acid Number"** or proportion of free fatty acid in the fat, estimated by titration in alcoholic solution; the **"Reichert-Meissl Number,"** or proportion of volatile fatty acids yielded by distilling the hydrolyzed fats with steam; the **Saponification-value** or milligrammes of potassium hydroxide neutralized by the saponification of one gramme of the fat; and the **Acetyl Number**, or amount of acetic acid yielded by 1 gramme of fat after treatment with hot acetic anhydride. In the following table the melting-points, iodine numbers and saponification-values of some of the fats most commonly employed are enumerated:

Fat.	Melting-point.	Iodine number.	Saponification value.
Butter-fat	28°–33° C.	26– 38	220–233
Pork-fat	36°–46° C.	50– 70	195–197
Beef-fat	40°–48° C.	36– 48	193–200
Sheep-tallow	44°–49° C.	33– 46	192–195
Human fat	17.5° C.	57– 66	193–199
Cod-liver oil	0°–10° C.	144–168	175–193
Cotton-seed oil	3°– 4° C.	105–117	191–196
Olive oil	2°–10° C.	78– 91	185–194
Linseed-oil	−27° C.	173–202	190–195

Cod-liver Oil is of especial interest to the physician because of its widespread employment as a food and therapeutic agent in chronic wasting diseases such as tuberculosis, and rickets. It is obtained from the livers of codfish by extraction with steam and water. It consists of a mixture of the glycerides of a great variety of saturated and unsaturated fatty acids together with a considerable proportion of phospholipins, a small amount of cholesterol, numerous nitrogenous bases and traces of iron, manganese, bromine and iodine. The therapeutic value of the oil has been variously attributed to each of these constituents in turn, and on the other hand to the readily digestible character of the oil itself. Modern opinion inclines to the view that the efficacy of cod-liver oil resides mainly in its high calorific value, and the fact that it is usually added in considerable dosage to the preestablished dietary. On the other hand it is very rich in accessory foodstuffs and the possible significance of some of these must not be overlooked. From this point of view it is not impossible that the therapeutic applications of cod-liver oil may be destined to increase rather than to diminish, as our growing knowledge of the exact require-

ments of the tissues enables us to use it with more judgment and less empirically than heretofore.

Cotton-seed Oil consists of a mixture of the glycerides of oleic and linoleic and palmitic acids, while **Olive Oil** consists almost entirely (89 per cent. to 98 per cent.) of the triglyceride of oleic acid.

Linseed-oil is of very great importance in the industries on account of its peculiar property of hardening when it dries in thin films exposed to the air, forming a transparent waterproof surface and accelerating the drying of other substances (pigments, etc.), with which it is mixed. This process of hardening takes place at first slowly, and then more rapidly, the products of oxidation which are formed accelerating the further stages of the process. The oxidation of linseed-oil which results in hardening is, in fact, an "autocatalytic," that is, a self-accelerated reaction, producing its own catalyzers. These substances are believed to be unstable peroxides which readily break down, liberating oxygen or possibly ozone, which oxidizes adjacent molecules of the oil. Other substances which accelerate the hardening are powdered lead, zinc, copper, platinum or their oxides. This phenomenon depends upon the very large proportion of unsaturated linkages which linseed oil contains; it consists of a mixture of the glycerides of linoleic, linoleinic and isolinoleinic acids (fatty acids of the unsaturated series $C_nH_{2n-6}O_2$) with a small proportion of oleic, palmitic and myristic acids, and a trace of unsaponifiable material.

Castor-oil is obtained by expression from the seeds or "beans" of the castor-oil plant (*Ricinus communis*). It consists in the main of the glycerides of ricinoleic acid $C_{17}H_{32}(OH).COOH$, a hydroxy-acid of the unsaturated series. It is without aperient action until saponified by the bile and pancreatic juice in the upper part of the small intestine and is therefore devoid of irritant action upon the walls of the stomach.

WAXES.

In addition to the glycerides of fatty acids there are found in a variety of living tissues and tissue-products, fatty acid esters of monatomic alcohols which are collectively and somewhat loosely designated **Waxes.** This term is not infrequently extended to include the cholesterol esters of the fatty acids, for no better reason than that cholesterol is a monatomic alcohol, and that the cholesterol esters somewhat resemble the waxes in certain of their properties, more particularly in the difficulty with which they are saponified. It would be preferable, however, to restrict the term "wax," as we are doing here, to the fatty acid esters of the higher monatomic alcohols of the paraffin series. In this way we will include in the class all of the most typical waxes of commerce, and we will exclude the entirely atypical esters of cholesterol.

The waxes are characterized by their high melting-point and the difficulty with which they are saponified. They are not hydrolyzed

by the fat-splitting ferments (lipases), and it is only with comparative difficulty that they are split into their components by alkalies. They are hydrolyzed by bacteria, and hence do not turn sour or "rancid" on standing. While their high melting-points prevent them from being "sticky" or exhibiting any of the characteristic properties of fluids or oils at ordinary temperatures, yet they retain the "greasy" or slippery qualities of the fats (more accurately expressed as the possession of a low coefficient of friction), and their insolubility in water, a combination of qualities which renders them ideal and indeed indispensable agents for polishing and waterproofing the surfaces of rough or porous materials.

In the skull of the white whale or cachelot (*Physeter macrocephalus*), there is found a large cavity which during life, is filled with an oily liquid. This liquid partially solidifies after the death of the animal, and consequent fall in temperature, and separates into two portions, a solid crystalline part ordinarily called **Spermaceti** and a liquid known as **Spermaceti-oil**. Spermaceti is also found in some other whales and in certain species of dolphins.

Purified spermaceti is a mixture of fatty acid esters of monatomic alcohols. The chief constituent is the palmitic acid ester of **Cetyl Alcohol**, $C_{16}H_{33}OH$, mixed with small quantities of the lauric, myristic and stearic acid esters of the twelve, fourteen and eighteen carbon atom alcohols of the paraffin series (general formula $C_nH_{2n+1}OH$).

Spermaceti is used for making "wax-candles," as a finishing material or waterproof polish, and in pharmacy as a means of stiffening emollients and salves, and raising their melting-point, particularly in hot climates. Spermaceti-oil is a very valuable lubricant for small and delicate machinery or apparatus.

The **Beeswax** of commerce is a digestion-product of the honey-bee, *Apis mellifica*. It is elaborated by special glands and the production of honey and wax stand in inverse proportion to one another, the production of one gramme of wax diminishing the yield of honey by from ten to fourteen grammes. Regarding the mode of origin of the wax from the foodstuffs of the bee, we are wholly in the dark.

The chief constituent of beeswax is the palmitic acid ester of **Myricyl Alcohol** $C_{30}H_{61}OH$ with an admixture of other acids and esters. Beeswax is employed in a variety of industries too numerous to mention here, it is an important constituent of a variety of commercial waxes, which are prepared by the admixture of paraffin and other substances with the beeswax to obtain the combination of physical qualities which is desired for the purposes for which the wax is to be employed. Adulteration with paraffin and other non-saponifiable materials may be detected by the low saponification-value of the mixture, the saponification-value of pure beeswax lying between 90 and 97.

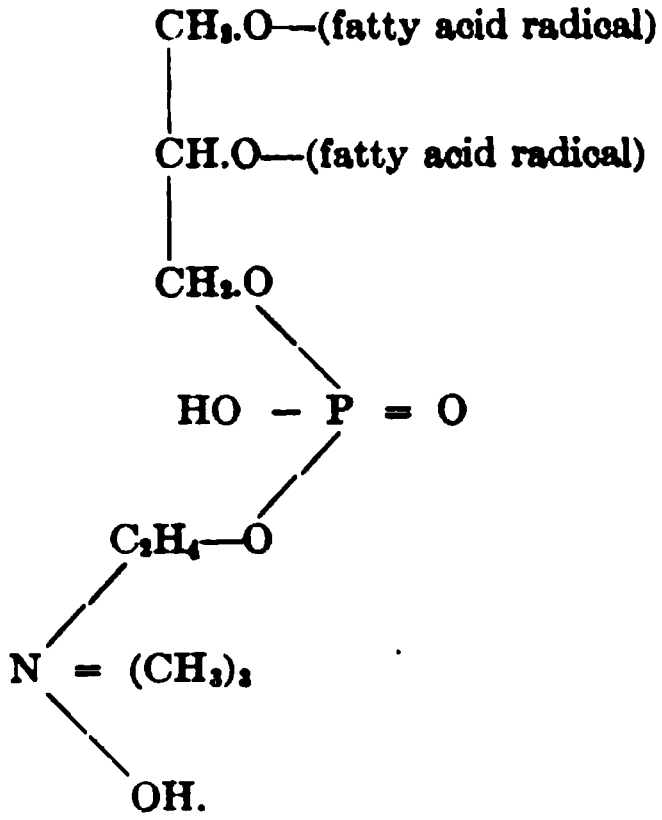
Waxes are produced by a variety of insects, notably the *Hymenoptera* (wasps and bees) and *Homoptera* (cicadas and scale insects). **Japan Wax** (or **Chinese Wax**) is obtained from a scale-insect which

infests the Chinese Ash. The most widely employed vegetable wax is carnaüba wax, obtained from the leaves of the Wax-palm or Carnaüba-palm which grows in tropical South America. It consists of a complex mixture of esters of higher monatomic alcohols.

THE PHOSPHOLIPINS OR PHOSPHATIDS.

In all living tissues and without exception, we find a variety of complex substances resembling the fats in their solubility in organic solvents, and yielding fatty acids, alcohols (usually glycerol), phosphoric acid and nitrogenous bases when hydrolyzed. These substances constitute the group of **Phospholipins**, and on account of their constant association in the tissues with cholesterol and cholesterol derivatives they are sometimes included with these in the larger group of **Lipoids** or fat-resembling substances, the common characteristic of the group consisting in their high solubility in fats and oils, and in the various fat-solvents. The term *lipoid*, however, is merely a convenient brief designation of a heterogeneous group of substances which may be chemically unrelated to one another. The phospholipins, on the contrary, are a rather well-defined and homogeneous group of chemically related substances.

The best known and most abundant representatives of the phospholipin group are the **Lecithins**. These substances, which are found in every living cell, yield fatty acids, glycerol, phosphoric acid and choline (=oxyethyl trimethyl ammonium hydroxide) on hydrolysis. One molecule of phosphoric acid is yielded for every molecule of choline, and the phosphorus and nitrogen-contents of these substances stand therefore in the proportion to one another of 1 : 1. The structure of the lecithins is believed to be represented by the formula:



The fatty acid radicals consist, as a rule, of palmitic, stearic or oleic acid, but at least one oleic acid radical would appear to be invariably present, since the lecithins exhibit to a very high degree the character-

istic instability of the unsaturated fatty acids. This instability is in fact enhanced in the phospholipins generally to a remarkable degree, and the difficulties attending their preparation and purification are rendered exceptionally great by their extreme susceptibility to oxidation. It is a fact which is doubtless of very great significance that the tissues of the **Brain** are notably rich in phospholipins, while the activities of the brain are exceptionally dependent upon an abundant and continuous supply of oxygen, the first bodily activities to disappear in asphyxia being those of the higher cerebral centers.

The various members of the phospholipin group resemble one another very closely in physical and chemical behavior. They differ among themselves mostly markedly, *first* in the proportion of phosphorus to nitrogen which they contain, and *secondly* in their solubilities in certain organic solvents.

Those phospholipins which, like lecithin, contain one atom of phosphorus (*i. e.*, one molecule of phosphoric acid) for every atom of nitrogen, are termed **Monoamino-monophosphatids**; those which contain two molecules of phosphoric acid for every atom of nitrogen (P : N = 2 : 1), are termed **Monoamino-diphosphatids**; those which contain two atoms of nitrogen for every atom of phosphorus are termed **Diamino-monophosphatids** (P : N = 1 : 2), and so forth. The highest proportion of nitrogen to phosphorus which has been found to occur in a phosphatid is that of four atoms of nitrogen for every atom of phosphorus.

The majority of the phospholipins are soluble in alcohol and in ether, but some of them are insoluble in ether, and others, while soluble in alcohol or in ether alone, are insoluble, or but sparingly soluble in certain mixtures of the two. The great majority of the phospholipins, but not all of them, are precipitated from ether solutions by the addition of acetone, a fact which is utilized very frequently in their preparation. They are also precipitated by a variety of metallic salts, and platinum chloride, and particularly cadmium chloride are frequently employed for their separation and purification.

The phospholipins are amorphous substances which are generally white or cream-colored when pure, but darken rapidly on exposure to the air. The iodine-number simultaneously diminishes, indicating that the unsaturated linkages have been partially neutralized by combination with oxygen. This oxidation is particularly accelerated by heat and by traces of moisture, and the dried or partially dried phospholipins are unfortunately extremely hygroscopic, rapidly attracting and condensing moisture when exposed to the air. The drying of phospholipins without decomposition can therefore only be achieved at low temperatures, and *in vacuo* or in an atmosphere composed of some indifferent gas. There seems to be some reason for supposing that the lability of the phospholipins may be greatly enhanced by impurities which are commonly associated with them, and that in the absence of these, they may be comparatively stable.

The majority of the phospholipins are rapidly hydrolyzed by the fat-splitting ferments or **Lipases**, yielding fatty acids, glycero-phosphoric acid and nitrogenous bases. Glycero-phosphoric acid is not split by lipase, but is readily decomposed by dilute acids yielding phosphoric acid and glycerol. Since glycero-phosphoric acid is not liberated from phospholipins until they reach the small intestine, where the reaction is alkaline, it would appear unlikely that it is split before absorption. According to some authors, an enzyme exists in tissue extracts from the liver, kidneys and intestinal mucosa, which is capable of bringing about the decomposition of glycero-phosphoric acid, but the constant presence of undecomposed glycero-phosphoric acid in small amounts in normal urine would appear to render this doubtful. Optically inactive glycero-phosphoric acid is readily prepared synthetically from glycerol, and phosphoric acid; the glycero-phosphoric acid yielded by hydrolysis of phospholipins is, however, levorotatory. It is soluble in water and insoluble in alcohol. The calcium salt is readily soluble in cold, but almost insoluble in boiling water.

The **Lecithins**, the composition and probable structure of which, have already been discussed, occur in all plant and animal cells. A lecithin or a mixture of lecithins, is particularly abundant in the yolks of eggs, and may be obtained in impure condition by extracting the broken yolks with ether, and adding acetone to the extract. Lecithins are particularly abundant in young and rapidly growing or embryonic tissues. They progressively diminish as development proceeds and, in the embryos of sea-urchins at all events, they are probably the source from which the phosphoric acid is obtained which is required to build up the nucleic acids in the nuclei of the new cells. The lecithins are soluble in alcohol, ether, chloroform, carbon bisulphide, benzol, and fats or fatty oils; they are precipitated from ether by the addition of acetone. In water they swell up and form pasty masses which throw out oily drops and threads, the so-called "myelin forms," into the body of the liquid. This probably represents the beginning of an imperfect emulsification, and the addition of soaps, saponins or bile-salts accelerates and completes the process with the formation of relatively stable milky emulsions which are coagulated by the addition of small quantities of salts of the alkaline earths (calcium, barium or strontium). The lecithins have a peculiar greasy odor which is rather sharp and reminiscent of dried brain-tissue. They are tasteless.

It was formerly believed that lecithins stood in a peculiar relationship to certain types of snake-venom and other hemolyzing poisons. If a pure hemolyzing snake-venom be allowed to act upon thoroughly washed blood-corpuscles, no hemolysis occurs. Upon the addition of blood-serum or of impure lecithin, which by themselves are of course without action, hemolysis ensues at once. It has been ascertained by Bang, however, that pure lecithin prepared from yolk of eggs is devoid of activating influence upon cobra-venom. It would thus appear probable that the activating action of other preparations of

lecithin is attributable to impurities which may nevertheless be phospholipins. Similarly the alleged action of lecithin in "activating" fibrin-ferment (the blood-coagulating ferment), has recently been shown to be due, in fact, not to lecithin but to kephalin.

In egg-yolk and in a variety of tissues we find another mono-amino-monophosphatid, designated **Kephalin** which differs from lecithin in being insoluble in alcohol. It is particularly abundant in brain-tissue and may be extracted by dehydrating the tissue with acetone, extracting with ether and adding alcohol to the concentrated extract. It may be purified by precipitation with cadmium chloride. It used to be alleged that kephalin differed from lecithin only in the addition of a methyl group, but recent investigations have shown the difference to be much more profound, inasmuch as the nitrogenous base in kephalin is not cholin, but amino-ethyl alcohol. Besides its great abundance in brain-tissues, where it doubtless plays an important rôle. Kephalin is of very great physiological importance in consequence of the part which it plays in the coagulation of the blood. The recent investigations of Howell and McLean have shown that kephalin possesses in high degree the power of neutralizing the anti-thrombin in the blood and thus permitting the thrombin, or fibrin-ferment to transform fibrinogen into fibrin and bring about coagulation of the blood. Very small amounts of kephalin, therefore, decisively accelerate the coagulation of the blood and kephalin is now being utilized extensively for this purpose in surgery.

Cuorin which is found in heart-muscle is a mono-amino-diphosphatid (P : N = 2 : 1). It is insoluble in alcohol and in acetone, but very soluble in ether, chloroform and benzol. It readily emulsifies in water and on the addition of alkalies the emulsions form clear solutions. The nature of the nitrogenous base in cuorin is not known. It contains three fatty acid radicals for every two molecules of phosphoric acid.

Sphingomyelin, which is found in the brain, is a diamino-monophosphatid. It is soluble in hot alcohol but sparingly soluble in cold alcohol, and insoluble in ether. It yields on hydrolysis an alcohol of unknown structure, **Sphingol** instead of glycerol, and it yields two different nitrogenous bases, namely **Neurine** and a base of which the structure is not yet completely defined, which is designated **Sphingosine** (see p. 197).

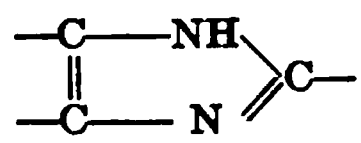
GLUCOSIDES OF THE PHOSPHOLIPINS.

If glucose be added to an ether solution of lecithin, the sugar, which is ordinarily insoluble in ether, dissolves and becomes so closely associated with the lecithin that repeated precipitation and resolution do not remove it. We infer that lecithin forms a compound with glucose, which, however, would appear to be a very loose one, since the analyses of various preparations indicate a very inconstant composition.

In the tissues of the liver we find considerable quantities of a water-soluble phospholipin, **Jecorin**, which yields glucose, fatty acids, glycerophosphoric acid, choline and hydrogen sulphide on hydrolysis by alkalies. Early observers were inclined to regard jecorin simply as lecithin glucoside, but the sulphur-content of jecorin precludes the adoption of this simple interpretation. Jecorin, like other phospholipins, is exceedingly hygroscopic and susceptible to oxidation. It is soluble in alcohol containing water and in ether, but is insoluble or but sparingly soluble in absolute alcohol. With silver nitrate an aqueous solution of jecorin yields a precipitate which dissolves in excess of the jecorin solution; on the addition of ammonia this mixture turns dark red. It appears highly probable from the variability of the analytical data obtained with jecorins from different sources that a variety of substances of this general type exist in the tissues; more especially is this indicated by the variable ratio of phosphorus to nitrogen which in some preparations approximates to the value P : N = 1 : 2, while in others P : N = 1 : 4. In any case it is probable that the phospholipin portion of the molecule is not simply lecithin. All preparations contain sodium, which appears to be present in chemical combination.

In the anterior lobe of the pituitary gland is found another water-soluble phospholipin which, however, instead of yielding glucose on hydrolysis, yields the cyclose **Inosite**. This substance, **Tethelin**, is soluble in water, alcohol or ether, but is insoluble in a mixture of alcohol and ether in the proportion of one part by volume of alcohol to one and one-half parts by volume of ether. It contains phosphorus and nitrogen in the proportion of 1 : 4 and one-half of the nitrogen is present in the molecule in the form of amino-groups. On hydrolysis the yield of amino-nitrogen increases to three-fourths of the total nitrogen, indicating, probably, that one of the nitrogen atoms is present in the form of an imino-group (= NH).

Tethelin is exceedingly hygroscopic and susceptible to oxidation which is accelerated by traces of moisture. Oxidation is accompanied by progressive darkening of the originally cream-colored powder, so that it ultimately becomes dark brown or almost black; at the same time the iodine absorption number decreases. With a 2 per cent. solution of p-dimethylamino-benzaldehyde in hydrochloric acid of sp. gr. 1.09, aqueous solutions of tethelin yield a pink coloration (**Ehrlich's Reaction**, indicating an acetylated oxyamino-acid radical). The nitrogenous base has not as yet been identified, but it probably contains an **Iminazolyl** radical:



This is of great significance in view of the fact that the active principle of the **Posterior Lobe of the Pituitary Body** is also an iminazolyl derivative and the possibility is indicated, which the anatomical

relations of the two glands tend to confirm, that the active principle of the posterior lobe is derived by partial decomposition from that of the anterior lobe.

Tethelin, in very small dosages administered by mouth, has a remarkable effect upon the growth of animals, consisting in the main of an initial retardation followed by a notable acceleration of growth. These phenomena will fall under fuller consideration in a later chapter. When administered locally in aqueous solution or incorporated with lanoline and applied as a salve it very markedly accelerates the repair of slowly healing lesions of the skin, such for example as various ulcers. The effects of tethelin upon growth almost exactly reproduce those of the whole anterior lobe tissue, and it is inferred that tethelin is the active principle, the absence or superabundance of which is responsible for the remarkable clinical manifestations of hyperactivity (gigantism and acromegaly) of the pituitary gland.

Tethelin in aqueous solution has no effect upon the uterus, and only causes in very large dosages a slight transient fall in blood pressure when injected intravenously in animals. After acidifying the solution (or rendering alkaline), heating for a brief period to nearly boiling-point and then cooling and neutralizing, however, the solution has a powerful effect in causing tonic contractions of the uterus similar to those caused by extracts of the posterior lobe, and when injected intravenously causes the characteristic rise in blood pressure which is brought about by small doses of posterior lobe extract (**Pituitrin**).

In the tissues of the brain we find a complex substance or series of substances which arise by combination of phospholipins with the cerebrosides which are glucosides yielding galactose on hydrolysis. This substance, **Protagon**, may be obtained by extracting dehydrated brain-tissue (dehydrated by acetone) with 85 per cent. alcohol, and cooling to zero when the protagon in impure form is precipitated. On partial hydrolysis it yields sphingomyelin (see p. 116) and cerebrosides (see p. 91).

Protagon when dry forms a fine white powder, it dissolves in 85 per cent. alcohol at 45° C., but on cooling is precipitated in groups of small acicular crystals. It is difficultly soluble in cold alcohol or ether, but dissolves in warm ether. It dissolves in methyl alcohol containing chloroform but, on standing, this solution decomposes and a cerebroside, **Phrenosin** is deposited. In water protagon swells up and forms a pasty mass which dissolves in excess of water, forming an opalescent solution. Solutions of protagon in pyridin are dextrorotatory, but on standing they decompose, depositing sphingomyelin, and become levorotatory.

We are not certain whether protagon is a chemical individual or not. The composition as reported by different observers, varies very considerably, and yet preparations have been obtained which failed to alter in composition or optical rotation of their solutions after repeated resolution and recrystallization.

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CHAPTER VII.

THE PROTEINS AND THE AMINO-ACIDS.

GENERAL CHARACTERISTICS OF THE PROTEINS.

THE greater and most characteristic part of the organic matter in protoplasm consists of colloidal substances containing nitrogen which are designated **Proteins.** As examples of the proteins we may recall white of egg, which is practically a solution of protein in dilute sodium chloride solution, or casein, which is flocculated out of milk by the addition of acids; and gelatin, which is derived from the connective tissues by extraction with hot water.

The proteins all contain carbon, hydrogen, nitrogen and oxygen, while the great majority of them also contain sulphur and a very great many of them contain phosphorus. Other constituents, for example, iron, copper and iodine are found in certain exceptional proteins or in compounds of the proteins with non-protein radicals containing these elements. The average composition of the more typical proteins is represented in the following table:

Element.	Per cent.
C	50.6 to 54.5
H	6.5 to 7.3
N	15.0 to 17.6
S	0.3 to 2.2
P	0.4 to 0.9
O	21.5 to 23.5

When perfectly free from water, the proteins form loose white powders, but when imperfectly dry, and especially if exposed to heat, they tend to form horny semi-transparent flakes or plates, so that in most of the older literature, before the modern methods of dehydration at low temperature by absolute alcohol and ether were employed, the proteins are usually described as horny substances when in the dry condition.

While drying, and in the presence of traces of moisture the proteins show a marked tendency to discoloration, with the production of heavily pigmented insoluble substances which are probably related to the "humin-substances" which are produced in the presence of carbohydrates by boiling the tryptophane radical of proteins with acids. Many proteins have curious and characteristic faint odors, but they are generally tasteless and amorphous.

Notwithstanding their colloidal character and very slight diffusibility in solutions, many proteins may, nevertheless, under suitable

conditions, be obtained in crystalline condition. This is particularly true of hemoglobin, of egg-albumin, the serum-albumin of the horse, and a variety of vegetable proteins. The solutions of the proteins are always optically active and with the exception of the solutions of hemoglobin and the nucleo-proteins, are levorotatory.

The great majority of the proteins are soluble in water or in very dilute acids or alkalies. Some exceptional proteins, however, such as **Elastin** from elastic fibers of connective tissues, **Keratin** from horn, **Fibroin** from silk and **Spongin** from sponges, are insoluble in water or in dilute acids and alkalies and require strong acids or alkalies to bring them into solution. The action of strong soda upon a sponge may be cited in illustration. The proteins are usually insoluble in organic reagents, although some of the vegetable proteins, particularly those obtained from a variety of grains, are soluble in 80 per cent. alcohol. Many of the proteins not commonly regarded as alcohol-soluble are, however, soluble in faintly alkaline alcohol, if they are first dissolved in alkaline water, and alcohol added up to 80 or 90 per cent. Casein is soluble in warm anhydrous formic acid, but the protein undergoes decomposition if the solution is allowed to stand.

The proteins combine with both acids and bases, neutralizing them wholly or in part, and causing a diminution of hydrogen ions in the case of combination with acids, or of hydroxyl ions in the case of combination with bases. They therefore belong to the class of substances designated **Amphoteric Acids**, or acids which are simultaneously capable of acting as bases. Under certain conditions the proteins are also capable of combining with neutral salts.

When dissolved in water, especially in faintly acid solutions, the proteins are usually modified by heat in such a way as to render them less soluble. This generally leads to flocculation or **Coagulation** of the protein, or if the solution be very concentrated, to the formation of a firm jelly, such as, for example, the white of a hard-boiled egg.

The true characterization of the proteins depends upon the presence among their hydrolytic cleavage products of a preponderating proportion of **Amino-acids.** No other single "test" can be relied upon to demonstrate the presence of a protein in a solution containing unknown substances, nor can the individual proteins be accurately characterized, as a general rule, in any other terms than the proportions of various cleavage-products which they yield on hydrolysis. By employing a multiplicity of tests, however, the presence of protein in a solution may be established by the fact that the unknown substance yields several positive reactions. For the identification of any particular protein we depend upon slight peculiarities of solubility in various salt solutions, dilute acids and alkalies, etc., and upon physical peculiarities and the nature of the tissue or fluid in which the protein occurs.

The various reactions which the majority of the proteins yield may be subdivided into **Coagulation-reactions** which involve or depend

upon dehydration of the protein and the formation of complex insoluble anhydrides, **Precipitation-reactions**, which depend upon the formation of insoluble compounds with the precipitating-agents employed, and **Color-reactions** which depend upon chemical interaction with the reagents employed, resulting in the production of distinctive colors. The most important of these reactions are the following:

COAGULATION-REACTIONS.

1. **Heat.**—Heat applied to solutions acidified with acetic acid. If mineral acids are employed, compounds of the protein with the acid may be formed which are incoagulable by heat.

2. **Alcohol.**—Alcohol added to neutral or acid solutions.

3. **Concentrated Neutral Salts.**—Concentrated neutral salts, particularly ammonium sulphate or magnesium sulphate. In acidified solutions concentrated sodium chloride or sodium sulphate are also coagulants of protein.

4. **Strong Mineral Acids.**—Upon the ability of the strong mineral acids to coagulate proteins depends **Heller's Test** for protein in urine. The suspected sample of urine is placed in a test tube and concentrated nitric acid is allowed to flow into the bottom of the tube from a pipette. At the junction of the two fluids a white ring of coagulated protein is formed.

Precipitation Reactions.—1. The **Salts of Heavy Metals**, such as cupric sulphate, lead acetate, mercuric chloride, silver nitrate, etc., form insoluble compounds with proteins. In the presence of excess of the reagent, the precipitate which at first forms not infrequently redissolves.

2. The so-called **Alkaloidal Reagents**, such as phosphotungstic or phosphomolybdic acids, tannic acid, potassium mercuric iodide, picric acid, trichloracetic acid, phenol and salicyl-sulphonic acid. Other reagents which similarly precipitate proteins are metaphosphoric acid, nucleic acids, chondroitin-sulphuric acid and taurocholic acid. Potassium ferrocyanide and acetic acid yield an insoluble compound of the protein with hydroferrocyanic acid.

Color-reactions.—1. **Millon's Reaction.**—A solution of mercury in strong nitric acid yields a mixture of mercuric and mercurous nitrates dissolved in a mixture of nitric and nitrous acids. If this reagent be added to a protein solution, a precipitate is produced which turns brick-red on heating.

2. **The Xanthoproteic Reaction.**—On adding strong nitric acid to protein solutions and heating to boiling, a pale yellow solution or coagulum results. On rendering the mixture alkaline with ammonia it becomes orange-yellow.

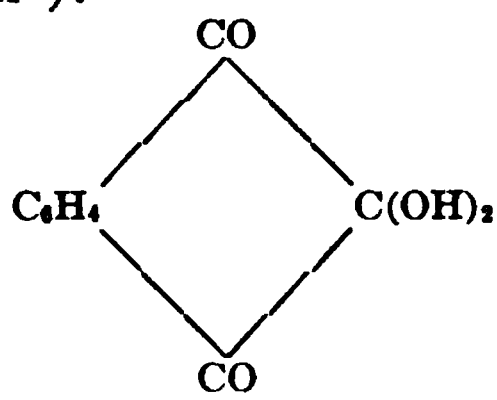
3. **The Hopkins-Cole Reaction.**—A solution of glyoxylic acid, formed by acting upon a concentrated solution of oxalic acid with sodium amalgam, is added to the protein solution in a test tube, and sulphuric

acid introduced at the bottom of the tube by means of a pipette. A reddish-violet ring is formed at the junction of the two liquids.

4. **Acree's Reaction.**—To the solution is added an equal volume of a 0.02 per cent. solution of formaldehyde containing a trace of ferric chloride. Concentrated sulphuric acid is then introduced below the mixture and at the junction of the two fluids a violet ring is formed.

5. **The Biuret-reaction.**—The protein solution is rendered strongly alkaline with concentrated sodium or potassium hydroxide, and a dilute solution of cupric sulphate is added, one drop at a time. A reddish or bluish violet results in solutions of proteins, and a pink color in solutions of their digestion-products, the peptones. Exceptions are afforded by the protamine group of proteins, which yield a pink biuret-reaction without preliminary hydrolysis.

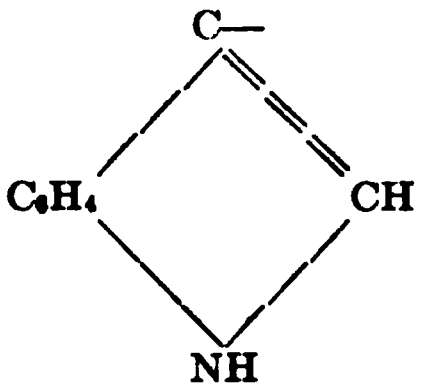
6. **The Ninhydrin Reaction.**—One-tenth of a gram of **Triketohydrindenehydrate** ("Ninhydrin"):



is dissolved in from thirty to forty c.c. of water, one or two drops of this solution are added to one c.c. of the protein solution, and the mixture is heated for a short time to boiling. On cooling, an intense blue or bluish violet color develops. This reaction is given not only by proteins, but also by their cleavage-products, proteoses, peptones and even amino-acids, with the exception of proline and oxyproline. This reaction is exceedingly delicate and is given by substances containing at least one free carboxyl-group, and one amino-group; it will detect glycine (amino-acetic acid) in solutions containing only one part in ten thousand. This extreme delicacy, in fact, renders the reaction rather unserviceable as a practical test for proteins or their decomposition-products, since such extraordinary precautions have to be taken to ensure that a positive test may not have been attributable to accidental contamination of reagents or apparatus with traces of the many substances that will yield a positive reaction.

All of these color reactions, with the exception of the biuret reaction and the ninhydrin reaction, depend upon specific atomic groupings which are usually, but not invariably present in the protein molecule. Thus, Millon's reaction is attributable to a hydroxy-benzene radical which is usually present in proteins in the form of the amino-acid **Tyrosine**, but is absent from certain proteins, for example gelatin. The xanthoproteic reaction is attributable to aromatic groups which are provided by the **Tyrosine**, **Phenylalanine** and **Tryptophane** radicals in the protein molecule. The xanthoproteic reaction is therefore not given by proteins such as the members of the protamine group in which

these radicals are lacking. The Hopkins-Cole reaction is attributable to the indole linkage:



which is present in the tryptophane (indole aminopropionic acid) radical of protein. It is therefore not given by proteins from which this radical is absent, such as, for example, zein, a protein obtained from corn (maize).

The biuret-reaction, on the contrary, is yielded by a variety of substances such as oxamide, biuret, etc., which are not proteins. The **Deaminized Proteins** which are laboratory-products formed by acting upon proteins with nitrous acid, and in which the NH_2 groups have been replaced by hydroxyl-groups, no longer give the biuret-reaction, although they otherwise resemble the natural proteins in physical and chemical behavior.

THE CLASSIFICATION OF THE PROTEINS.

American and English biochemists have unfortunately adopted slightly different systems of classification of the proteins. The American classification, adopted by the American Physiological Society and the American Society of Biological Chemists, is as follows:

I. SIMPLE PROTEINS:
- **Albumins.**
- **Globulins.**
- **Glutelins.**
- **Prolamins (alcohol-soluble proteins).**
- **Albuminoids.**
- **Histones.**
- **Protamines.**

II. CONJUGATED PROTEINS:
- **Nucleoproteins.**
- **Glycoproteins.**
- **Phosphoproteins.**
- **Hemoglobins.**
- **Lecithoproteins.**

III. DERIVED PROTEINS:

1. **Primary Protein Derivatives:**
   - **Proteans.**
   - **Metaproteins.**
   - **Coagulated Proteins.**
2. **Secondary Protein Derivatives:**
   - **Proteoses.**
   - **Peptones.**
   - **Peptides.**

The classification adopted by the British Medical Association is the following:

I. SIMPLE PROTEINS:
- **Protamines.**
- **Histones.**
- **Albumins.**
- **Globulins.**
- **Glutelins.**
- **Alcohol-soluble Proteins.**
- **Scleroproteins.**
- **Phosphoproteins.**

II. CONJUGATED PROTEINS:
- **Glucoproteins.**
- **Nucleoproteins.**
- **Chromoproteins.**

III. PRODUCTS OF PROTEIN HYDROLYSIS:
- **Infraproteins.**
- **Proteoses.**
- **Peptones.**
- **Polypeptides.**

Neither of these systems of classification is free from objection. To them both the general objection applies, that the distinctions drawn are largely based upon variations in physical behavior which do not necessarily correspond to fundamental differences of chemical architecture while, on the other hand, many protein or protein-like substances are known which display intermediate characteristics, or individual peculiarities which render their inclusion in any of the classes enumerated, a matter of more or less arbitrary opinion. In particular the defect of the American system lies in the rather intangible distinctions which are made between various classes of primary protein derivatives, and the inclusion of coagulated proteins which are almost certainly derived from native proteins by abstraction of water from the molecule, in the same class with proteans and metaproteins which are derived from native proteins by partial hydrolysis, is unfortunate. The English classification has the merit of simplicity, but it would be more advisable to include the phosphoproteins among the conjugated proteins, as in the American classification, and to add a fourth group to accommodate the coagulated proteins. The term prolamine is also preferable to the designation "alcohol-soluble proteins," because it draws attention to the high content of the amino-acid proline which characterizes these proteins more fundamentally than the physical property of solubility in eighty per cent. alcohol.

The salient characteristics of these various classes of protein substances are as follows:

I. THE SIMPLE PROTEINS.

Protamines.—The protamines are the simplest proteins which are as yet definitely known to occur in nature. They are found in spermatozoa, and especially in the spermatozoa of fishes in combination with nucleic acid, forming a simple type of nucleoprotein. They are predominantly basic substances, indeed so strongly basic that a solution of salmine (the protamine from salmon spermatozoa) reacts alkaline to litmus and absorbs carbon dioxide from the air, forming carbonates of the protamine. The acid function of these substances is correspondingly weak, although they are, like all proteins, amphoteric acids, and in the presence of excess of strong bases will partially combine with them.

The protamines are soluble in water and form definite salts with acids which are coagulated by alcohol and thrown out of solution without decomposition, the combined acid being carried down quantitatively with the protein. They yield a pink biuret-reaction resembling in this respect the derivatives of the partial hydrolysis of other native proteins. They yield, when completely hydrolyzed, a preponderating proportion of diamino-acids.

Histones.—The histones are somewhat more complex and colloidal in character than the protamines, and their basic function is less marked. They are still predominantly basic, however, and occur in cellular tissues, combined with nucleic acid, and in the chromoprotein, hemoglobin, combined with a colored acid radical, **Hematin.** They are soluble in dilute acids or dilute solutions of the strong bases, but are precipitated from acid solutions by the addition of ammonia.

Albumins.—The albumins are markedly colloidal substances which are soluble in distilled water and in salt solutions. The basic function is almost equal to the acid function. Representative examples are egg-albumin and the albumin which is found in blood-serum. They are coagulated by saturation of their solutions with ammonium sulphate.

Globulins.—The globulins are very decidedly colloidal substances passing, for example, with difficulty, or not at all through clay filters. They are insoluble in distilled water, but are soluble in dilute solutions of strong acids or bases, or of inorganic salts. The acid function predominates slightly over the basic, so that they neutralize bases more readily and completely than acids. Typical examples are afforded by serum-globulin, the globulin which is precipitated from egg-white by dilution with distilled water, and a variety of vegetable proteins such as edestin, obtained from seeds of hemp (*Cannabis Sativa*). They are coagulated by half-saturation of their solutions with ammonium sulphate or complete saturation with magnesium sulphate.

Glutelins.—The glutelins are a group of vegetable proteins of which only two, the **Glutenin** of wheat and the **Oryzenin** of rice have as yet been prepared in sufficient quantity, and purity to render analysis and characterization possible. They are insoluble in water or dilute

salt solutions but they are soluble in dilute solutions of strong bases or acids.

Prolamins.—The prolamins are soluble in 70 per cent. to 90 per cent. alcohol. They are insoluble, or nearly so, in distilled water, but dissolve readily in dilute solutions of strong acids or bases. They occur in a variety of grains, typical members of the group being **Gliadin**, found in the seeds of wheat and rye, **Hordein** found in the seeds of barley and **Zein** found in the seeds of maize. They are characterized by the high proportion of **Proline** which they yield when hydrolyzed.

Scleroproteins.—The scleroproteins, termed albuminoids in American and Continental European publications, form a very heterogeneous group of substances. The various proteins which we have hitherto been considering are either constituents of cellular tissues, concerned in the life and maintenance of the protoplasm, or else they form reserve-materials which are sooner or later to be called upon to supply the requirements of protoplasm. Quite other is the function of the scleroproteins, for these are proteins of a primarily structural or architectural rather than nutritional significance. They are binding, cementing and supporting substances which contribute in a mechanical rather than in a chemical fashion to the furtherance and maintenance of life. They occur especially in the various connective tissues, and corresponding with their peculiar function, we find that they display a variety of physical characteristics, distinguishing them from the proteins of cellular origin, and also distinguishing the individual members of the group very sharply from one another. Typical members of this class are **Gelatin** and its parent-substance **Collagen** which forms the chief constituent of white fibrous connective tissue, and also the main organic constituent of bones. On boiling, especially in the presence of dilute acid, **Collagen** yields the cleavage-product **Gelatin.** Collagen itself is insoluble in water, salt solutions and dilute acids or alkalies, but gelatin swells in cold water and dissolves in warm water, forming jellies on cooling if the solutions are sufficiently concentrated. **Reticulin,** occurring in the reticular fibrous tissues of glands differs from collagen in several respects, notably in containing phosphorus.

Keratin is another scleroprotein and forms the chief constituent of the horny epidermal structures, hair, wool, nails, hoofs, horns, feather, tortoise-shell, etc. A form of keratin, **Neurokeratin**, also occurs in nervous tissues. Keratin is insoluble in water, dilute acids or alkalies and salt solutions; it is soluble with difficulty in strongly alkaline solutions. It is also characterized by the high percentage of sulphur which it contains and which is attributable to the amino-acid radical **Cystine.**

Elastin forms the chief constituent of the elastic fibers of connective tissue. It is distinguished by its elasticity and tensile strength and also by its extreme insolubility, being soluble only in strong caustic alkalies or concentrated mineral acids. **Fibroin,** the substance forming

the core of silk fibers, is characterized by possibly even greater tensile strength, while it is somewhat more readily dissolved by concentrated acids and alkalies than elastin. **Sericin** or silk gelatin forms the outer coating of the silk fiber, and is sticky when freshly secreted, so that it enables intersecting and adjacent fibers to adhere. It is soluble in hot water, and the solution resembles a solution of ordinary gelatin in that, if concentrated, it gelatinizes on cooling. Finally, **Spongin** forms the chief part of the ordinary sponge from which the originally living protoplasm has been extracted. It is insoluble in acids but soluble in concentrated alkalies. Some of the spongins contain iodine as an integral part of the molecule.

The scleroproteins are for the most part incomplete proteins in the sense that they do not yield when completely hydrolyzed, all of the amino-acids that we are accustomed to obtain from the more typical proteins of cellular tissues. Thus gelatin yields neither tyrosine nor tryptophane, elastin and fibroin yield neither aspartic nor glutamic acids, and spongin yields neither tyrosine nor phenylalanine.

The extraordinary variety of physical properties and peculiarities displayed by the various scleroproteins reveals the possibility of substances of very unique physical characteristics being derived from proteins, and would point to the ultimate possibility of very important industrial applications of such derivatives. At the present time, horny derivatives of the protein of milk, casein, are extensively used in the manufacture of substitutes for ivory, celluloid and bone. The animal proteins, being among the most expensive foodstuffs we require, can never be employed very extensively in the industries, excepting when they form by-products of the foodstuffs-industry, as in the manufacture of glue from slaughter-house or fish-wastes, and of casein products from skimmed milk. Certain vegetable proteins might, however, be rendered relatively cheap and abundant and offer an interesting field for the investigation of the special physical characteristics of their derivatives.

II. THE CONJUGATED PROTEINS.

Nucleoproteins.—The conjugated proteins are complex substances formed by the union of a protein with a non-protein radical, which may be termed the **Prosthetic Group**. The **Nucleoproteins**, for example, are compounds of **Nucleic Acids**, which are substituted phosphoric acids containing carbohydrate and nitrogenous radicals, with a protein which plays the part of a base in the compound. These compounds are the most characteristic constituents of the nuclei of cells. When the protein constituent is a histone, the compound is termed a **Nucleohistone**.

The nucleoproteins are insoluble in distilled water, but soluble in dilute alkalies from which solutions they are precipitated by weak acids, such as acetic acid or carbon dioxide. They are as a rule incompletely digestible by the pepsin of gastric juice, leaving an indigestible

residue which still contains protein and is termed **Nuclein.** All of the nucleoproteins appear to be very closely associated, or possibly combined with **Iron.**

Glucoproteins.—In the glucoproteins the prosthetic group is either, an amino-carbohydrate, a polysaccharide derived from glucosamin or acetylated derivatives of glucosamin, or else chondroitin-sulphuric acid (see p. 91). The glucoproteins are subdivided into **Mucins, Mucoids** and **Chondroproteins.** The true mucins yield extraordinarily glutinous or mucilaginous solutions from which the mucin is precipitated by acetic acid. The mucoids are not precipitable by acetic acid and do not, as a rule, yield such highly viscous solutions as the mucins. The chondroproteins are insoluble in water, but are soluble in dilute alkalies, from which solutions the protein is precipitated by neutralization with strong acids or by an excess of acetic acid. They yield **Chondroitin-sulphuric Acid** on hydrolysis, a substituted sulphuric acid formed by the union of a molecule of **Chondroitin** with a molecule of sulphuric acid. Chondroitin resembles gum-arabic in physical characteristics, and is a compound of **Glucuronic Acid** and **Glucosamin.** The mucins occur in mucous secretions, as for example the secretions from the skin-gland of snails or slugs. Mucoids are found in connective tissues, in the vitreous humor of the eye and in the white of egg (ovomucoid). The chondroproteins occur especially in cartilaginous tissues, and in the interstitial substance of connective tissue. Chondroproteins are also found in the accumulations of colloidal material which characterize the "amyloid degeneration" of certain organs under pathological conditions.

Phosphoproteins.—The phosphoproteins are proteins which yield phosphoric acid when hydrolyzed. The most typical example of this group is **Casein,** the chief protein constituent of milk, but phosphoproteins also occur in a variety of vegetable tissues, and in the yolk of egg (ovovitellin). They are predominantly acid in character, as might be expected, not only from their content of phosphoric acid, but also from the fact that they yield a high proportion of dicarboxylic amino-acids on hydrolysis.

Chromoproteins.—The chromoproteins are compound proteins in which the prosthetic group is colored. The most typical examples of the group are **Hemoglobin,** the red coloring-matter and oxygen-carrier of blood, in which the prosthetic group is a complex iron-containing organic acid **Hematin,** and **Hemocyanin,** a blue pigment containing copper which plays a rôle corresponding to that of hemoglobin in the *Arachnidæ* and *Crustacea.* The chromoproteins, hemoglobin and hemocyanin, are exceptional among proteins in the relative ease with which they are obtainable in crystalline condition. The protein radical in hemoglobin is a predominantly basic protein, known as **Globin** and related to the histones.

Lecithoproteins.—The lecithoproteins are compound proteins in which the prosthetic group is a phospholipin. This is rather a con-

jectural group of substances, for although proteins associated with phospholipins have been prepared from yolk of egg, and from vegetable tissues, it is not yet certain whether the phospholipin is an integral part of the protein molecule, or merely a contamination which is physically adherent to it. Evidence of an electrochemical character has demonstrated, however, that compounds between lecithin and proteins are formed when the two substances are mixed in aqueous solution, and we may infer that similar compounds may not improbably exist in nature.

III. THE PRODUCTS OF PROTEIN HYDROLYSIS.

Infraproteins.—The infraproteins are substances produced in the initial stages of protein hydrolysis which still retain the characteristic properties of the proteins. Examples are the **Acid-** and **Alkali-albuminates**, formed from albumins by gentle heating in acid or alkaline solutions, and which differ from albumins in being insoluble in neutral distilled water. Other examples are **Paracasein**, formed by the action of rennet or weak pepsin upon casein, and the **Paranucleins** which are formed by the partial digestion of a variety of phosphoproteins.

Proteoses.—The proteoses are hydrolytic cleavage-products of the proteins which have lost the characteristic protein property of being coagulable by heat, but they retain the coagulability by ammonium sulphate. They are usually subdivided into **Primary Proteoses** which are coagulable by half-saturation of their solutions with ammonium sulphate, and **Secondary** or **Deuteroproteoses** which are coagulated by complete saturation of their solutions with ammonium sulphate. The majority of the proteoses are coagulable by alcohol, but certain of them are soluble in alcohol. They yield a reddish violet or pink biuret-reaction.

A considerable number of the proteoses are toxic when injected into the circulation, while the native proteins with a few marked exceptions, such as the **Ricin** in castor-oil beans (*Ricinus Communis*) are non-toxic. On the other hand the native proteins are **Antigenic** that is, they give rise, on repeated injection into the circulation of animals, to substances which circulate in the blood serum and have the property of precipitating the particular protein against which the animal has been immunized. The proteoses on the contrary are as a rule non-antigenic.

Peptones.—The peptones are still simpler products of the hydrolytic cleavage of proteins. They are slightly diffusible, and they are incoagulable either by heat or by ammonium sulphate. They are, however, precipitable by tannic acid, phosphotungstic acid or lead acetate. They are usually coagulable by alcohol, although certain peptones, especially when combined with acid, are not coagulable by alcohol. They yield a clear pink biuret-reaction, and are non-antigenic and, as a rule, non-toxic.

IV. THE COAGULATED PROTEINS.

The coagulated proteins may be subdivided into two classes, namely, those in which the coagulation-process has gone so far as to be *irreversible*, so that the coagulum cannot be brought back into solution again without preliminary decomposition into simpler substances, and those in which the coagulum remains soluble after removal of the coagulating-agent and in which the coagulation-process has therefore remained *reversible*. The majority of the heat-coagulated proteins belong to the first class, although the incipient stages of heat-coagulation are sometimes reversible. On the other hand the coagula produced by alcohol or by ammonium sulphate belong to the second class, although in some instances after more or less prolonged contact with alcohol the coagula produced by alcohol become irreversible.

The polypeptides or chains of amino-acids out of which proteins are built up, form anhydrides with exceptional ease, either by internal neutralization of carboxyl- and amino-groups, or by the condensation of several molecules, and this tendency increases with increasing length of the amino-acid chain. We can hardly suppose, therefore, that this property has been lost in the much more bulky amino-acid complexes which constitute the proteins. On the other hand the agencies which bring about coagulation are all of such a character (heat, alcohol and concentrated salts) as to suggest that the withdrawal of water from the protein is the chemical basis of the coagulation-process. It appears very probable, therefore, that coagulation is due to the formation of protein anhydrides, and that the irreversible coagula are those in which the anhydride-formation has proceeded furthest.

THE END-PRODUCTS OF PROTEIN HYDROLYSIS: THE AMINO-ACIDS.

Decomposition of proteins into simple crystallizable substances may very readily be brought about by a variety of agencies, but the only methods of decomposition which yield easily interpretable results are those which bring about **Hydrolysis**, or decomposition of the molecule by successive splittings with the addition of the elements of water. Hydrolysis of the proteins (autohydrolysis) will take place spontaneously in neutral protein solutions or even if precipitated proteins be left in long-continued contact with neutral and sterile water. The process is, however, greatly accelerated by the application of heat, especially by temperatures considerably exceeding the temperature of boiling water, or by catalyzers, of which the most efficient are acids, alkalies and the protein-digesting (proteolytic) enzymes. Whatever the means of hydrolysis employed, however, the end-result, provided the hydrolysis has been complete, is the same, namely, the production of a mixture of amino-acids.

Incomplete hydrolysis, however, results in the production of a number of intermediate substances, variously designated, in the order of decreasing complexity, **Proteoses (Albumoses), Peptones, Polypeptides and Dipeptides.** The hydrolysis of the proteins, therefore, occurs in stages, just as, in the hydrolysis of starch, intermediary stages (the dextrins and maltose) are passed through before the attainment of the last stage of hydrolysis and the quantitative conversion of the starch into glucose.

It is not certain, however, whether the various intermediate products of protein hydrolysis represent successive stages of hydrolysis or whether in some instances comparatively simple products may not be split off from the proteins or proteoses, leaving complex residues, so that complex and simple intermediate substances are produced simultaneously. Probably both types of cleavage occur at different points in the protein molecule. Those linkages which are most accessible to the action of the particular catalyzer employed will be disrupted first, and if some of them chance to lie near the extremities of the molecule, simple products and a complex residue will result, while disruption of more internal linkages will break the molecule into parts of more equal weight and complexity.

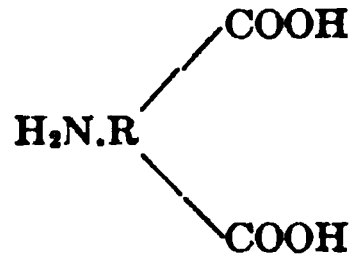
It was early recognized that amino-acids form the chief part of the decomposition-products which result from the hydrolysis of protein. The separation of the individual amino-acids from one another, and their quantitative estimation, was a much more difficult matter. The first attempts to isolate individual amino-acids from the mixture which the complete hydrolysis of a protein yields, depended upon the fractional crystallization, either of the free amino-acids or of their salts. Except in the case of the very slightly soluble amino-acids, such as tyrosine, these methods were not even approximately quantitative, and even the isolation and identification of a given amino-acid could only be effected with certainty when that acid was present in relatively large amounts. The attainment of our present relatively extensive knowledge of the nature and quantities of the amino-acids which result from protein hydrolysis, is an achievement of the past twenty years, and we owe it in the first place to the investigations of Kossel and of Emil Fischer and their pupils.

The various amino-acids which are yielded by the proteins are limited in number, and probably do not exceed eighteen or nineteen. These however, fall into several very distinct classes, namely:

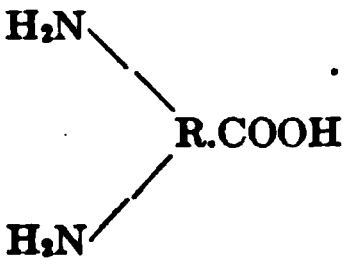
Monoamino-monocarboxylic acids, general formula:

$$H_2N.R.COOH$$

Monoamino-dicarboxylic acids, general formula:



Diamino-monocarboxylic acids, general formula:



Diaminohydroxy-monocarboxylic acids.

Heterocyclic compounds, *i. e.*, amino-acids containing a ring of atoms, one or more nitrogen atoms being included in the ring.

The first necessary step in the analysis of the amino-acids produced by the hydrolysis of protein, consists in the separation of the diamino-acids from the monoamino-acids. This may be accomplished by means of phosphotungstic acid, which precipitates the diamino-acids while the monoamino-acids are left in solution. The diamino-acids **Arginine** and **Histidine** may be separated in the form of their silver salts, while the remainder of the precipitate produced by phosphotungstic acid consists of the diamino-acid **Lysine.**

The monoamino-acids may be estimated by evaporating the whole mixture to dryness *in vacuo* and then dissolving the mixture of acids in alcohol, and passing into the solution dry hydrochloric acid gas which catalyzes the formation of alcohol esters of the amino-acids. These esters are volatile and may be separated into fractions each containing only a small number of acids, by means of fractional distillation *in vacuo*. The esters in each fraction are then reconverted into free acids and alcohol by hydrolysis, and the individual acids separated and estimated by special methods adapted to the peculiar properties of each of the acids present in the particular fraction concerned. The difficultly soluble acids, **Tyrosine, Cystine** and **Diamino-trioxydodecanic Acids** are separated from the digest before esterification.

This method, due to Emil Fischer, is laborious and inaccurate, but it greatly surpasses the methods which were formerly in use, and which did not even permit a partial separation of the various mono-amino-acids in a protein digest. The method permits the accurate quantitive determination of only five acids, namely, the diamino-acids histidine, arginine and lysine, and the monoamino-acids tyrosine and cystine. The estimates of the other acids are only approximate, and must be regarded as minimum values, since it has been found that in a known mixture of amino-acids it is only possible to account for about two-thirds of the nitrogen, by Fischer's method. For many purposes, in which a knowledge of the total proportion of nitrogen present in the form of monoamino-acids suffices, Fischer's method has now been largely superseded by the method of Van Slyke which is described below (p. 144), but if we desire to ascertain approximately the quantity of the individual monoamino-acids contained in a protein digest, Fischer's method, or modifications of it, still affords the only available procedure.

By these various methods the following amino-acids have been isolated from among the products of hydrolysis of various proteins:

A. Monoamino-monocarboxylic acids.

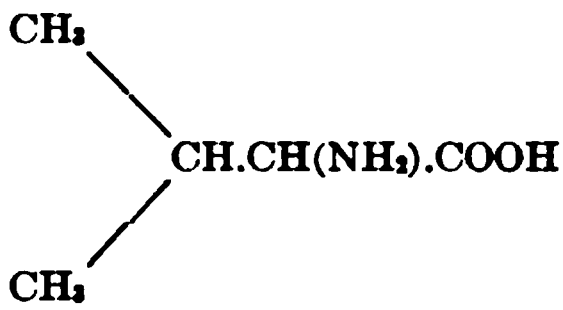
1. Glycine, or amino-acetic acid:

$$CH_2.NH_2.COOH$$

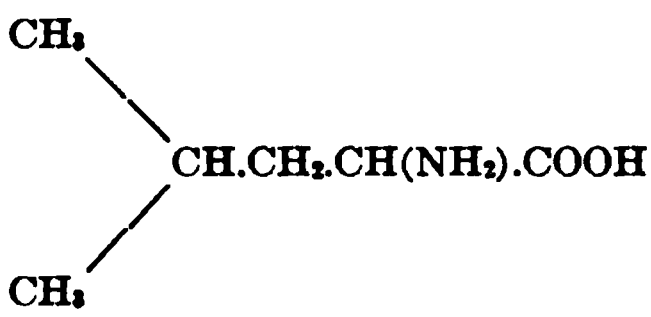
2. Alanine, or α-amino-propionic acid:

$$CH_3.CH(NH_2).COOH$$

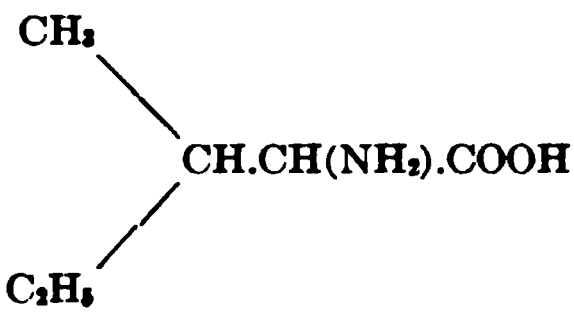
3. Valine, or α-amino-iso-valerianic acid:



4. Leucine, or α-amino-isocaproic acid:



5. Isoleucine, or α-amino-β-methyl-β-ethylpropionic acid:



6. Caprine, or Glycoleucine, or α-amino-normal-caproic acid:

$$CH_3.CH_2.CH_2.CH_2CH(NH_2)COOH$$

7. Phenylalanine, or β-phenyl-α-amino-propionic acid:

$$C_6H_5CH_2.CH(NH_2)COOH$$

8. Tyrosine, or β-parahydroxyphenyl-α-amino-propionic acid:

$$HO.C_6H_4.CH_2.C(NH_2).COOH$$

9. Serine, or β-hydroxy-α-amino-propionic acid:

$$CH_2(OH).CH(NH_2).COOH$$

10. Cystine, or dicysteine or di- (β-thio-α-amino-propionic acid):

$$HOOC.CH.(NH_2).CH_2S—SCH_2.CH(NH_2).COOH$$

B. Monoaminodicarboxylic acids.

11. Aspartic acid, or amino-succinic acid:

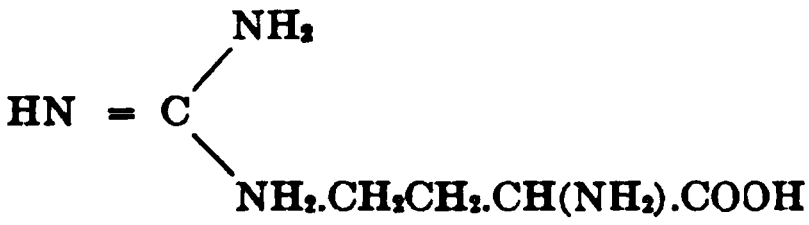
$$HOOC.CH_2CH(NH_2).COOH$$

12. Glutamic acid, or α-aminoglutaric acid:

$$HOOC.C_2H.CH_2CH(NH_2).COOH$$

C. Diaminomonocarboxylic acids.

13. Arginine, or α-amino-δ-guanidine-valerianic acid:

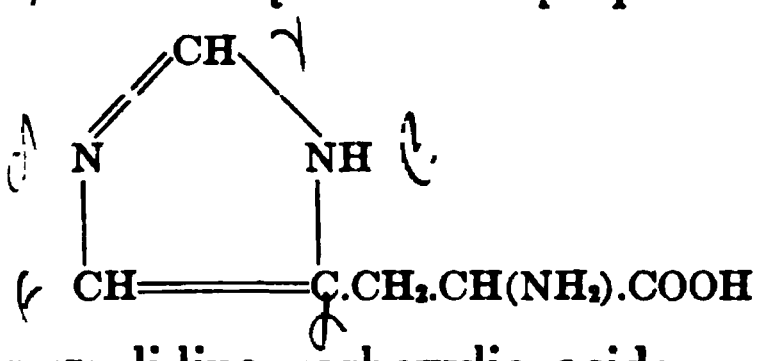


14. Lysine, or α-ω-diaminocaproic-acid:

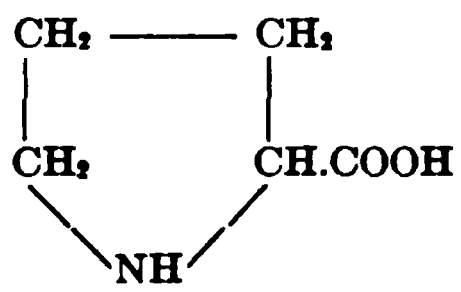
$$H_2N.CH_2.CH_2.CH_2.CH(NH_2).COOH$$

D. Heterocyclic amino-acids.

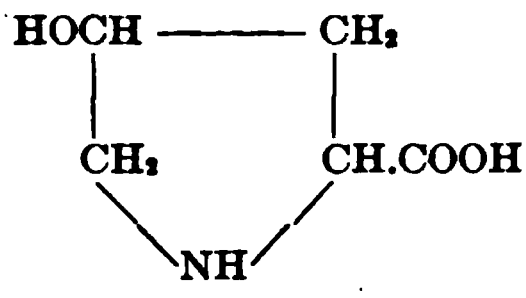
15. Histidine, or β-iminazolyl-α-amino-propionic acid:



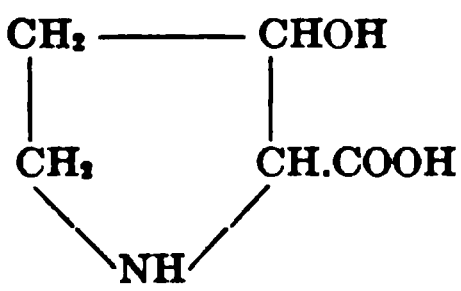
16. Proline, or pyrrolidine carboxylic acid:



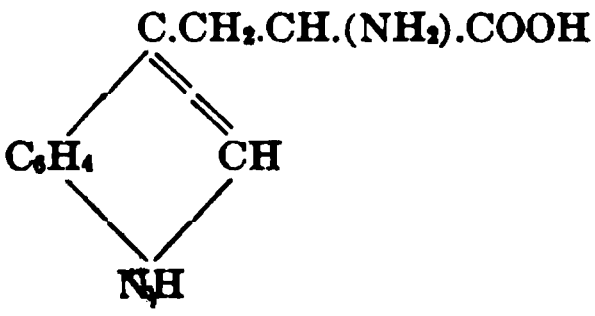
17. Oxyproline, or hydroxy-α-pyrrolidine-carboxylic acid:
Either:



or:



18. Tryptophane or β-indole-α-aminopropionic acid:



In addition to these acids two hydroxy-diamino-acids have been isolated from among the cleavage-products of one protein, namely, casein. The very great variety of proteins and protein derivatives which exist in nature are therefore constructed out of a relatively small and limited number of amino-acid building-stones, differing proportions and arrangements of these components being responsible for the wide variety of characteristics displayed by the native and derived proteins.

In many instances a definite parallelism can be traced between the chemical and physical behavior of the proteins and their amino-acid content. Thus, the **Albumins**, which are soluble in distilled water and are not coagulated by half-saturation of their solutions with ammonium sulphate, contain no glycine, while the **Globulins**, which (when uncombined with acids or bases) are insoluble in distilled water and are coagulated by half-saturation of their solutions with ammonium sulphate, do contain this amino-acid. The alleged transformation of serum albumin into globulin by warming in alkaline solutions observed by Moll, and not infrequently quoted, is therefore, an impossibility, since it would involve the synthesis of amino-acetic acid and its union with the albumin molecule which could not be brought about by any such simple procedure. The product actually obtained by Moll was an infraprotein, alkali-albuminate, which mimics globulin in being insoluble in neutral water, but differs from it in fundamental constitution.

The alcohol-soluble vegetable proteins (**Prolamines**) contain a trace (probably attributable to associated impurities) of glycine, and some of them contain no glycine, their content of diamino-acids is very small, while their content of glutamic acid and of proline is very high. The phosphoproteins, **Casein** and **Vitellin**, are also rather high in glutamic-acid content. **Gelatin** is characterized by its high glycine content and **Keratin** by its high content of cystine. The **Histones**, which are predominantly basic substances, contain about thirty per cent. of diamino-acids, while the **Protamines**, which are still more predominantly basic, contain only small amounts of monoamino-acids, **Salmine** (from salmon sperm) containing over eighty per cent. of arginine, while **Sturine** (from sturgeon sperm) contains sixty-seven per cent. of its nitrogen as arginine, ten per cent. in the form of histidine, and from six to seven per cent. in the form of lysine.

The amino-acids are white, crystalline, readily diffusible substances and the crystal form is characteristic for each amino-acid. The crystal forms of glycine, leucine and histidine are shown in the accompanying figures (3–5).

The amino-acids are usually readily soluble in water, cystine and tyrosine affording exceptions to this rule. They are, with the exceptions of proline and oxyproline, insoluble in alcohol and ether. They have high melting-points and melt with decomposition, splitting off carbon dioxide. With the exception of glycine the amino-acids are optically active, some of them being dextrorotatory and others levorotatory.

FIG. 3.—Glycine crystals. (After Hawk.)

FIG. 4.—Leucine crystals. (After Funke.)

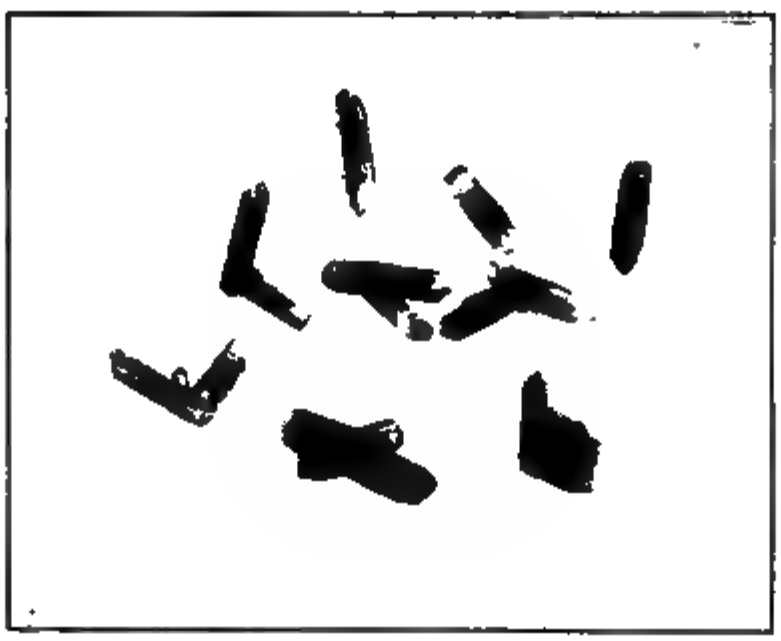


FIG. 5.—Histidine monochloride crystals.

The amino-acids, since they contain trivalent nitrogen and a carboxyl-group, are simultaneously bases and acids, in other terminology are **Amphoteric Acids.** They form crystalline salts with metallic bases and with mineral acids.

On treatment with nitrous acid the amino-acids lose their amino-group, which is replaced by a hydroxyl-group as follows:

$$\begin{matrix}CH_2.NH_2\\ |\\ COOH\end{matrix} + HNO_2 = \begin{matrix}CH_2OH\\ |\\ COOH\end{matrix} + N_2 + H_2O$$

It will be observed that all of the amino-acids obtained in the hydrolysis of proteins are α-amino-acids, that is, an amino-group is attached to the carbon atom immediately adjacent to the carboxyl-group. This is probably a fact of great significance, since, as we shall see, the proteins are formed by the union of long chains of amino-acids, linked together by means of their amino- and carboxyl-groups. These groups being closely adjacent, the resultant chains are shorter, and the weight of the other radicals in the molecule more evenly distributed than would be the case if the carboxyl- and amino-groups were separated by a long chain of carbon linkages, and the possibility of such heavy compounds as the proteins possessing sufficient stability to permit their formation probably resides in this device for shortening the chain of serial linkages. Corresponding to this view we find that the ω-amino-group which is also present in lysine, is not united in proteins to any carboxyl-group but remains free and reacts with nitrous acid just as the amino-acid does.

THE SYNTHESIS OF PROTEINS.

The marked predominance of amino-acids among the products of protein hydrolysis long ago led biological chemists to surmise that the amino-acid structure, or some derivative of that structure, must be represented in a high degree in the protein molecule, and it was in following up this clue that Schützenberger in 1888 carried out one of the earliest and most successful attempts to synthesize bodies of a protein character. Recognizing that the decomposition of proteins into amino-acids is essentially a phenomenon of hydrolysis, he regarded dehydration as an essential feature of any attempt at protein synthesis, while the abundance of amino-acids among the products of protein hydrolysis, and the presence therein, as he thought, of bodies related to urea, led him to believe that protein synthesis must consist in the linkage of amino-acids with molecules of urea and the elimination of water. Accordingly amino-acids were mixed with urea and phosphorus pentoxide and heated to 125° C. The product was a pasty solid, soluble in water and readily coagulated by alcohol. It was furthermore precipitated from aqueous solutions by the usual protein precipitants and gave the biuret- and xanthoproteic reactions.

This experiment of Schützenberger's left us, however, very much where we were, so far as real knowledge of the structure of the protein molecules is concerned. The knowledge of the fact that a mixture of amino-acids and urea yields, under certain treatment, a body or bodies more or less closely resembling the proteins, furnished us with little or no information regarding the structure of the protein molecule which we did not already possess in the fact that the disintegration-products of the proteins are predominantly amino-acids. Prior to Schützenberger, Grimaux, in 1881, had shown that condensation-products of aminobenzoic acid and aspartic acid resemble the proteins in many of their properties; but these experiments also threw no light upon the structure of the protein molecule beyond emphasizing the already sufficiently evident probability that the amino-acid grouping plays an important part in the building up of the protein molecule.

The clue which led, through a series of remarkable researches, to our present comparatively extensive knowledge of the groupings within the protein molecule, was obtained in 1883 by Curtius when he discovered that ethyl glycocollate (the ethyl ester of glycine) in watery solution tends to form **Glycine Anhydride**:

(In the absence of water)

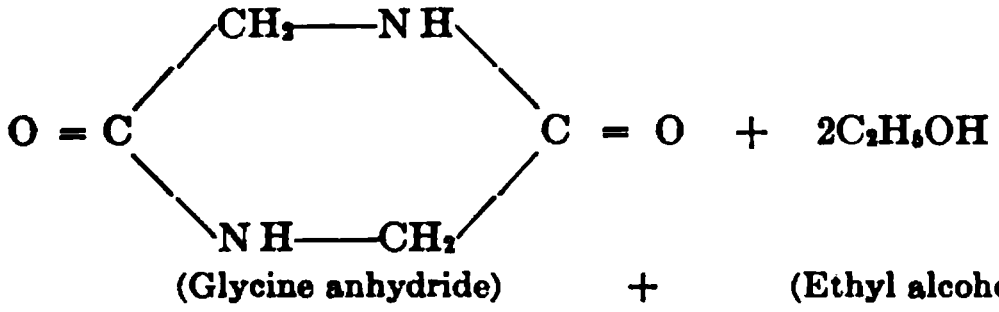
$$H_2N.CH_2.COOH + C_2H_5OH = H_2N.CH_2COOC_2H_5 + H_2O$$

(Glycine) + (Ethyl alcohol) = (Ethyl glycocollate).

(In the presence of water).

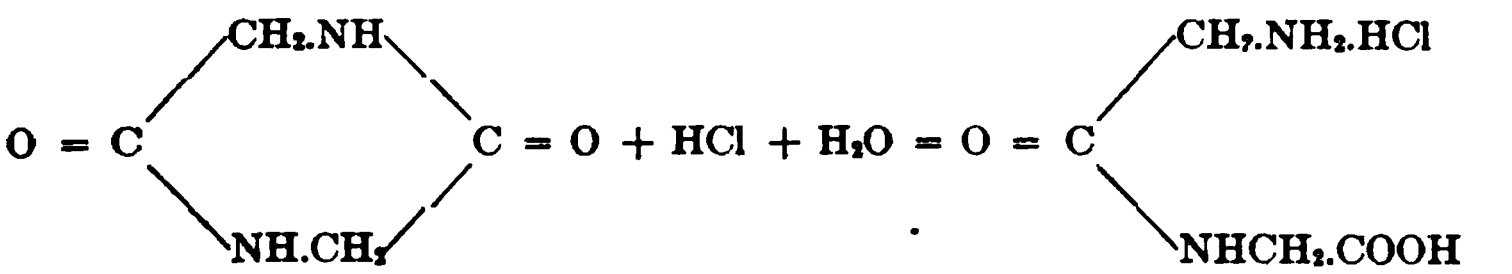
$$H_2N.CH_2COOC_2H_5 + H_2N.CH_2.COOC_2H_5 =$$

(Ethyl glycocollate) + (Ethyl glycocollate).



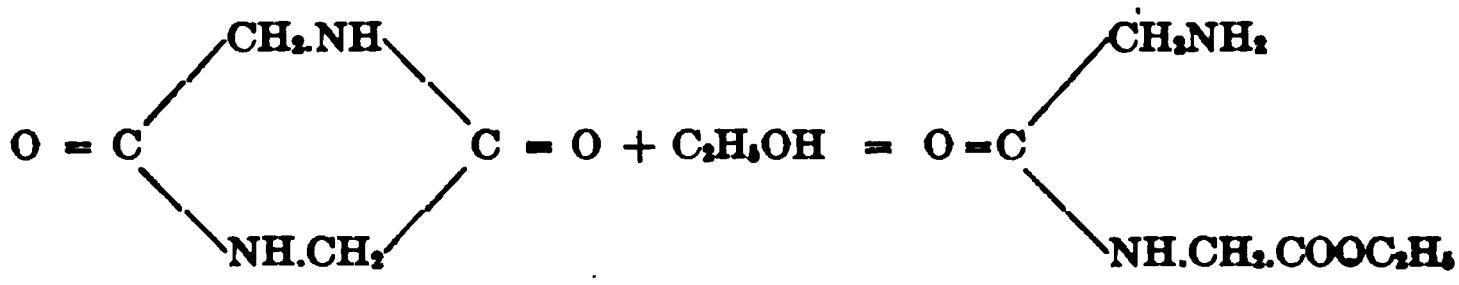
(Glycine anhydride) + (Ethyl alcohol).

Obviously, if the closed ring representing the glycine anhydride molecule could be opened up without destroying the stability of the molecule, a new amino-acid would be formed, one degree more complex than the original amino-acid (glycine). This possibility was realized by Emil Fischer, who found that if the glycine anhydride which is thus prepared be boiled for a short time with concentrated hydrochloric acid, the following change occurs:



(Glycine anhydride.) (Glycyl-glycine chloride.)

On now treating the glycyl-glycine chloride with silver oxide, silver chloride is precipitated and free **Glycyl-glycine** is obtained. If, however, the glycine anhydride be originally treated with *Alcoholic* instead of with an aqueous solution of hydrochloric acid, the ethyl ester of glycyl-glycine is obtained:



(Glycine anhydride.) (Glycyl-glycine ester.)

It would appear, therefore, as if we had only to repeat this cycle of operations indefinitely in order to secure the most complex poly-amino-acids; but this is not so easy as it might appear at first sight; the instability of polyamino-acids consequent upon the high reactivity of the $-NH_2$ group, and the consequent difficulty of obtaining simple anhydrides renders this procedure impossible. Moreover the anhydride-ring is in many cases (*e. g.*, leucine anhydride) very difficult to break up when it has once been formed.

In the search for methods of overcoming these difficulties Fischer found that the instability of the amino-acids could be eliminated by the introduction of radicals into the $-NH_2$ group, and he and Fourneau synthesized phenylcyanate-glycyl-glycine ($C_6H_5.NH.CO-NHCH_2CO-NHCH_2COOH$) and carboxethyl-glycyl-glycine ester ($C_2H_5O.OC.NH.CH_2.CO-NH.CH_2.COOC_2H_5$) which are both chemically stable bodies. In subsequent investigations Fischer found that, by gentle heating, combination between the esters of the carboxethyl-amino-acids and other amino-acids could be directly brought about, and in this way carboxethyl-diglycyl-leucine ester was formed:

$$C_2H_5OOC.NH.CH_2CO.NH.CH_2.CO.NH.CH.(C_4H_9)CO.OC_2H_5.$$

The difficulty was here encountered, however, that the carboxethyl-group having been once introduced, cannot be eliminated again.

The method which was devised to overcome this difficulty was extremely ingenious. The introduction of a radical into the $-NH_2$ group appeared to be a necessity, forced upon us by the impossibility of otherwise securing simple anhydrides of the acids. It occurred to Fischer, however, that the radical thus introduced into the $-NH_2$ group might itself be made a carrier of amino-acid groups into the molecule. This anticipation proved to be correct. The radical which was first utilized was the chloracetyl group ($ClCH_2.CO-$). When chloracetyl chloride is allowed to act upon glycyl-glycine ester (obtained by the methods described above), chloracetyl-glycyl-glycine-ester is obtained:

$$\underset{\text{Chloracetyl chloride)}}{ClCH_2COCl} + \underset{\text{(Glycyl-glycine ester)}}{H_2N.CH_2CO.NH.CH_2COOC_2H_5} =$$

$$ClCH_2.CO.NH.CH_2.CO.NH.CH_2.COOC_2H_5 + HCl$$

(Chloracetyl glycyl-glycine ester).

By saponification of this ester, free chloracetyl-glycyl-glycine is obtained. On now treating this with a concentrated aqueous solution of ammonia, the chlorine atom in the chloracetyl group becomes, by a usual reaction, replaced by an amino-group, and thus **Diglycyl-glycine** is obtained:

$$\underset{\text{(Chloracetyl glycyl-glycine ester)}}{ClCH_2.CO.NH.CH_2.CONH.CH_2COOC_2H_5} + 2NH_3 =$$

$$\underset{\text{(Diglycyl-glycine ester).}}{H_2N.CH_2.CONH.CH_2CONH.CH_2COOC_2H_5} + NH_4Cl$$

In other words, the chloracetyl-group, introduced to protect the $—NH_2$-group of the amino-acid is, after it has performed its protective function, itself transformed into an amino-acid-group, through the replacement of the halogen atom by $—NH_2$. Obviously, other halogen-containing acid groups may be used in place of chloracetyl, and in this way a great variety of amino-acid-groups can be introduced into the $—NH_2$-group. Among others the following are employed:

Chloracetyl chloride for the introduction of glycyl.

α-Bromopropionyl chloride for the introduction of alanyl.

α-Bromisocapronyl chloride for the introduction of leucyl.

α-Phenylbromopropionyl chloride for the introduction of phenyl-alanyl.

α-δ-Dibromovaleryl chloride for the introduction of prolyl.

By this method the chain of amino-acids is lengthened at the amino-group end. Theoretically it appeared possible to also lengthen the chain at the carboxyl-end of the molecule, by acting upon the esters of the amino-acids with the acid chlorides of other amino-acids. Until 1904, however, the acid chlorides of amino-acids were unknown, and all attempts to prepare them had failed, owing to the same reason which limits the use of the first method of synthesizing poly-amino-acids described above, namely the reactivity of the $—NH_2$-group. It will be recollected that Fischer found that the $—NH_2$-group could be protected by the introduction of radicals, and, utilizing this fact, in 1904 he succeeded in devising a method of preparing the acid chlorides of the amino-acids. The acid chlorides thus prepared, react with the esters of other amino- or polyamino-acids to form polyamino-acid chains of greater length. Thus:

$$\underset{\text{(Bromisocapronyl-glycyl chloride)}}{C_4H_9CHBr.CO.NH.CH_2.COCl} + \underset{\text{Glycine ester)}}{2NH_2.CH_2.COOC_2H_5} =$$

$$\underset{\text{(Glycine ester hydrochloride)}}{HClNH_2.CH_2.COOC_2H_5} + \underset{\text{(Bromisocapronyl-glycyl-glycine ester)}}{C_4H_9CHBrCONH.CH_2CO.NH.CH_2COOC_2H_5}$$

Subsequent saponification of the bromisocapronyl-glycyl-glycine ester and treatment with ammonia yields the polyamino-acid or (tripeptide), **Leucyl-glycyl-glycine**:

$$C_4H_9CH(NH_2)CO.NH.CH_2CONH.CH_2.COOH$$

If the bromisocapronyl-glycyl chloride be made to act upon glycyl-glycine ester, and the product treated with ammonia, the tetrapeptide **Leucyl-diglycyl-glycine** results:

$$C_4H_9.CH(NH_2)CO.NHCH_2.CO.NHCH_2CO.NHCH_2COOH$$

By these methods, and modifications of these methods, Fischer and others have succeeded in building up long chains of amino-acid-groups, these chains being collectively termed by Fischer, **Peptides.** Chains consisting of two links, *i. e.*, combinations of two amino-acids are designated **Dipeptides**; such, for example, are glycyl-glycine, alanyl-alanine and leucyl-leucine, chains consisting of three links are termed **Tripeptides**, such being, for example, diglycyl-glycine and leucyl-glycyl-glycine. Chains consisting of four links are termed **Tetrapeptides**, and so on, the higher members of the series being collectively termed **Polypeptides.**

The surpassing interest of these investigations lies in the fact that many of the polypeptides are considered to be, in all probability, identical with certain of the natural peptones derived from proteins by partial hydrolysis, while others probably merit inclusion among the proteins themselves. Thus the **Octadecapeptide**, l-leucyl-triglycyl-l-leucyl-triglycyl-l-leucyl-octaglycyl-glycine, and the **Tetradecapeptide**, l-leucyl-triglycyl-l-leucyl-octaglycyl-glycine so closely resemble, in general properties, the ordinary proteins, that they would undoubtedly have been classed among the proteins had they been first met with in nature. Thus, they give the biuret-reaction, and form opalescent watery solutions, and the tetradecapeptid is coagulated by ammonium sulphate and precipitated by tannic acid and by phosphotungstic acid. As they do not contain tyrosine, tryptophane, phenylalanine or cystine, they fail to give such protein color reactions as depend upon the presence of these groups.

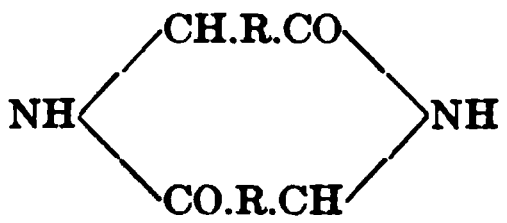
The molecular weight of the octadecapeptide is 1213, and the substitution of phenylalanine, tyrosine and cystine in the place of glycine groups would increase this weight two or three times, giving a value which is of the same order of magnitude as the more modern estimations of the (minimal) molecular or combining-weights of many of the natural proteins.

A whole series of the polypeptides give the typical peptone biuret reaction (pink), and such as contain tyrosine also give Millon's reaction. The biuret-reaction is, with the glycine compounds, first encountered in the tetrapeptide, but it is given by tripeptides built up to include other amino-acids. The biuret-reaction is, generally speaking, more intense the greater the length of the polypeptide chain.

The majority of the polypeptides are readily soluble in water, and such as are soluble in water with difficulty, are readily soluble in dilute mineral acids and alkalies, with which they combine; they are less soluble in solutions of acetic acid. As a rule they are insoluble in absolute alcohol, but in alcohol containing a little watery ammonia

they may be soluble, in which case they are precipitated on boiling off the ammonia.

Under conditions involving dehydration, as for example heating, or treatment of the esters with alcoholic ammonia, the dipeptides are converted into anhydrides which are ring-compounds, designated **Diketopiperazines**, and having the general formula:



Under similar conditions the polypeptides are modified in an analogous manner, yielding anhydrides with a ring-structure.

When hydrolyzed, the polypeptides break down into their constituent amino-acids, the imino-groups in the polypeptide molecule being converted, by taking up the elements of water, into amino-groups, the reaction being:

$$-COHN- + H_2O = -COOH + H_2N-$$

A very large number of the polypeptides are hydrolyzed by the protein-splitting enzymes, **Pepsin**, **Trypsin**, etc., and, in some cases, at all events, it is certain that the hydrolysis takes place in stages, as it does with the proteins and peptones. We will revert in more detail to this question in a later chapter (Chapter X).

THE OCCURRENCE OF PEPTIDES AMONG THE PRODUCTS OF PROTEIN HYDROLYSIS.

The fact that hydrolyzing agents, when they act upon proteins, lead to the production of the same substances, amino-acids, that the polypeptides yield when hydrolyzed, and the fact that the synthetic polypeptides resemble the native and derived proteins so closely in their physical and chemical characteristics, afford a very strong basis for the supposition that the proteins are, in fact, chains of amino-acid radicals, linked together as they are in the polypeptides by the mutual neutralization of $-NH_2-$ and —COOH-groups. Confirmation of this view is afforded by the fact that di,- tri- and tetrapeptides have been frequently found and identified among the products of the incomplete hydrolysis of proteins. Thus glycyl-d-alanine, glycyl-l-tyrosine, and a tetrapeptide built up by the union of two molecules of glycine, one of d-alanine and one of l-tyrosine have been isolated from among the cleavage-products of **Fibroin**, glycyl-l-leucine, l-leucyl-d-alanine, glycyl-d-valine and a dipeptide yielding d-alanine and l-proline on hydrolysis, have been obtained from **Elastin**, glycyl-l-proline has been obtained from **Gelatin**, and a lysyl-glycyl peptide from **Egg-albumin**. From **Gliadin** a dipeptide has been split off which yields phenylalanine and proline when completely hydrolyzed.

From the construction of these fragments of the protein molecule we may infer the architecture of the whole, and without going so far as to assume that no other types of linkage exist within the protein molecule, an assumption which would very possibly be incorrect, we may safely conclude that the —COHN-linkage plays a very predominant part in building together the constituent parts of the protein.

The tetrapeptide referred to above which has been isolated from the products of the incomplete hydrolysis of silk fibroin is of especial interest, because, had it not been identified as a tetrapeptide, it would certainly have been included among the proteoses. It is precipitated by phosphotungstic acid, readily dissolves in water, is insoluble in alcohol, and coagulated by saturation of its aqueous solutions with ammonium sulphate, or with sodium chloride. Its molecular weight, determined by the cryoscopic (freezing-point) method, was 350. The synthetic pentapeptide, l-leucyl-triglycyl-l-tryosine, possesses very similar properties, so that the proteoses are not necessarily exceedingly complex substances, nor is excessive complexity necessary in order that substances of this type may be coagulable by saturation of their aqueous solutions with ammonium sulphate.

THE ANALYSIS AND CHARACTERIZATION OF PROTEINS BY THE DETERMINATION OF THE AMINO-ACID RADICALS WHICH THEY CONTAIN.

The hydrolysis of the proteins is accompanied by a very marked increase in the total number of free amino-groups present in the solution. This is due to the fact that successive repetitions of the reaction:

$$—COHN— + H_2O = —COOH + H_2N—$$

result in the transformation of imino-groups into amino-groups, until the final number of amino-groups formed corresponds with the total number of amino-acid radicals out of which the protein is built up.

Reference has been made above to the fact that free amino-groups in the aliphatic series have the well-known property of reacting with nitrous acid, with the liberation of nitrogen, in accordance with the equation:

$$—RNH_2 + HNO_2 = ROH + H_2O + N_2$$

Very ingenious advantage has been taken of this fact by D. D. Van Slyke, in the method which he has devised, and which is now very widely utilized for the determination of the distribution and partition of nitrogen within the protein molecule.

This method consists essentially in the following process: The protein having been in the first place subjected to complete hydrolysis, the small proportion of ammonia which always occurs in the mixture of products (derived from "amide" nitrogen in the protein molecule) is first removed by vacuum-distillation and separately determined. The

residual mixture of products is then treated with phosphotungstic acid, which results in the precipitation of the diamino-acids, namely **Cystine, Arginine, Lysine** and **Histidine**, a determination of sulphur yields a measure of the cystine-content. Arginine has the property of yielding one-half of its nitrogen in the form of ammonia on boiling with alkali. The quantity of ammonia developed on boiling the precipitate with alkali therefore affords a measure of the content of arginine.

The total nitrogen in the phosphotungstic-acid precipitate is now determined, and from it is subtracted the calculated proportion of nitrogen which is contributed by the cystine- and arginine-content. The residual nitrogen is derived from lysine (= x) and histidine (= y). On treatment with nitrous acid lysine yields a volume of free nitrogen corresponding to the whole of its nitrogen-content (= x), while histidine yields a volume of free nitrogen which corresponds to one-third of its nitrogen-content ($=\frac{1}{3}y$). The amino-nitrogen content of the precipitate is therefore determined, by the nitrogen-yield on treatment with nitrous acid, and after subtracting the amino-nitrogen yield due to arginine (= one-fourth of the total nitrogen in arginine) and of cystine (= the whole of its nitrogen content) the residual amino-nitrogen evidently represents the whole of the lysine nitrogen plus one-third of the histidine nitrogen, or $x+\frac{1}{3}y$. But the determination of the *total* nitrogen in the phosphotungstic-acid precipitate, and the subtraction therefrom of the cystine and arginine nitrogen, has already given us a measure of the *total* nitrogen contained in the lysine and histidine, that is, of $x+y$. Subtracting, therefore, the amino-nitrogen yield due to these two amino-acids from the proportion of the total nitrogen which they contribute, the difference evidently corresponds to two-thirds of the histidine nitrogen, from which the contents of histidine and lysine may be readily computed.

In the filtrate from the phosphotungstic-acid precipitate the total nitrogen and the amino-nitrogen are separately determined. The difference yields a measure of the nitrogen contained in pyrrolidine (proline and oxyproline) or indole (tryptophane) rings.

For the determination of the nitrogen evolved from amino-groups on treatment with nitrous acid Van Slyke has devised a very convenient apparatus which permits the rapid and accurate determination of amino-groups in very small quantities of material. For descriptions of this apparatus the reader is referred to Van Slyke's original articles, or to current laboratory-handbooks.[1]

During the hydrolysis of proteins by hydrochloric acid a small amount of a very deeply colored precipitate separates out. The nitrogen-content of this precipitate is the so-called "humin" nitrogen. This appears to be derived from a portion of the tryptophane and, in the presence of a sufficiency of carbohydrate, which acts as a catalyzer for the formation of humin, the yield, of humin-nitrogen is

[1] For example R. H. A. Plimmer: Practical Organic and Biochemistry, London, 1915, p. 146.

stated to be a quantitative measure of the tryptophane-content of the protein.

The following are representative results obtained by Van Slyke's method.

PERCENTAGE OF THE TOTAL NITROGEN CONTAINED IN VARIOUS AMINO-ACID GROUPS OF THE UNDERMENTIONED PROTEINS.

	Gliadin.	Edestin.	Keratin (dog's hair).	Gelatin.	Fibrin.	Haemoglobin.
Ammonia N.	25.52	9.99	10.05	2.25	8.32	5.2
Humin N.	0.86	1.98	7.42	0.07	3.17	3.6
Cystine N.	1.25	1.49	6.60	0.00	0.99	0.0
Arginine N.	5.71	27.05	15.33	14.70	13.86	7.7
Histidine N.	5.20	5.75	3.48	4.48	4.83	12.7
Lysine N.	0.75	3.86	5.37	6.32	11.51	10.9
Mono-amino N.	51.98	47.55	47.50	56.30	54.30	59.0
Non-amino N. (= proline + oxyproline + ½ tryptophane N)	8.50	1.70	3.10	14.90	2.70	2.9
Total	99.70	99.37	98.85	99.02	99.58	100.04

The following are among the estimations of the amino-acids yielded by various proteins, estimated by the more exhaustive but less convenient method of Emil Fisher.[1] It must be recollected, however, that the estimates for the majority of the monamino-acids are minimal estimates only, for the reason that in the process of analysis a certain proportion of the nitrogen escapes estimation. It is probable, however, that in experienced hands the losses are of similar magnitude and kind so that the results obtained by different investigators may be considered comparable. From these figures it may clearly be seen how variable is the composition of different proteins in respect to their

[1] Cited from analyses reported by:
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Osborne, T. B., and Jones: Am. Jour. Physiol., 1910, **26**, p. 305.
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Levene and Beatty: Zeit. Physiol. Chem., 1906, **49**, p. 252.
Taylor, A. E.: Jour. Biol. Chem., 1908–9, **5**, p. 389.
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content of the various amino-acid radicals and how great a rearrangement and reassortment of radicals is necessary before one protein can be converted into another and, consequently, before the various proteins of the diet can be converted into the characteristic proteins of the various tissues of the body.

PROPORTION OF VARIOUS AMINO-ACIDS YIELDED BY CERTAIN PROTEINS ON HYDROLYSIS.

Amino acid.	Zein (maize), per cent.	Gliadin (wheat), per cent.	Edestin (hempseed), per cent.	Casein (milk), per cent.	Lactalbumin (milk), per cent.	Gelatin, per cent.	Protamin (salmin), per cent.	Ox muscle, per cent.
Glycine	0.0	0.0	3.8	0.0	0.0	16.5	0.0	2.1
Alanine	9.8	2.0	3.6	1.5	2.5	0.8	0.0	3.7
Valine	1.9	3.4	6.2	7.2	0.9	1.0	5.3	0.8
Leucine	19.6	6.6	20.9	9.4	19.4	2.1	0.0	11.7
Proline	9.0	13.2	4.1	6.7	4.0	7.7	10.8	5.8
Oxyproline . . .	?	?	2.0	0.3	..	6.4	0.0	..
Phenylalanine . .	6.6	2.4	3.1	3.2	2.4	0.4	0.0	3.2
Aspartic acid . .	1.7	0.6	4.5	1.4	1.0	1.2	0.0	4.5
Glutamic acid . .	26.2	43.7	18.7	15.6	10.1	1.8	0.0	15.5
Serine	1.0	0.2	0.3	0.5	?	0.4	8.7	?
Tyrosine	3.6	1.2	2.1	4.5	0.9	0.0	0.0	2.2
Cystine	?	0.5	0.3	0.1	?	0.0	0.0	..
Histidine . . .	0.8	2.2	2.4	2.5	2.1	0.4	0.0	1.8
Arginine	1.6	3.0	14.4	3.8	3.2	7.6	91.7	7.5
Lysine	0.0	1.2	1.7	6.0	9.2	2.8	0.0	7.6
Tryptophane. . .	0.0	1.0	..	1.5	..	0.0	0.0	..
Ammonia. . . .	3.6	5.2	2.3	1.6	1.3	..	..	1.1

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Van Slyke: Jour. Biol. Chem., 1909–10, **7**, p. xxxiv; 1911, **9**, p. 185; 1911–12, **10**, p. 15; 1915, **23**, p. 411; 1912, **12**, p. 295; 1913–14, **16**, pp. 121 and 125; 1915, **22**, p. 281; 1915, **23**, p. 407.

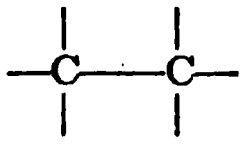
CHAPTER VIII.

COMPOUNDS OF THE PROTEINS.

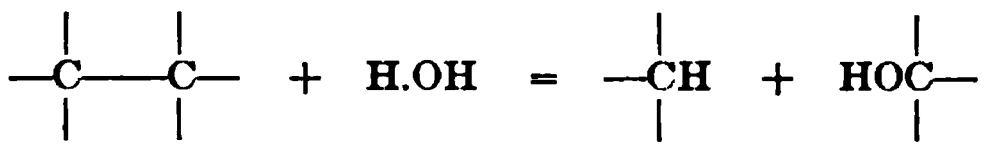
TYPES OF UNION IN THE PROTEIN MOLECULE.

Following the recognition of the fact that the proteins are complexes built up by the union of amino-acids, the question of the mode of union between them became one of paramount importance. Hofmeister has pointed out that it is possible to conceive of several ways in which amino-acids might be linked together, such as:

A. Direct union of the carbon atoms, as:

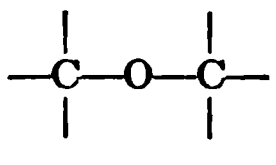


under which condition the molecule would be an immense chain of carbon atoms and if the addition of the elements of water (hydrolysis) were to accomplish the splitting up of the molecule, a large proportion of hydroxy-acids would result:



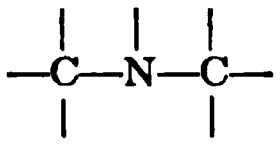
whereas hydroxy-acids (tyrosine, serine and oxyproline) form, as a rule, only a small proportion of the products of protein hydrolysis. Direct union of carbon atoms, therefore, cannot be a frequent mode of linkage of amino-acids within the protein molecule.

B. Ether-like unions, as:

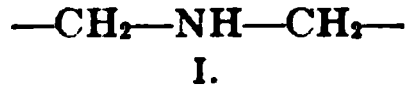


Such unions, however, would only be possible when one of the two amino-acids thus united contained a hydroxyl-group and, as we have seen, hydroxy-amino-acids constitute only a small proportion of the total amino-acids yielded by a protein when it is hydrolyzed.

C. The carbon atoms may be united through a nitrogen atom as:

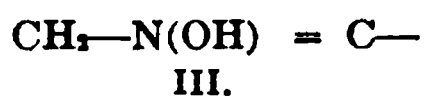


Several varieties of this mode of union are possible, as:

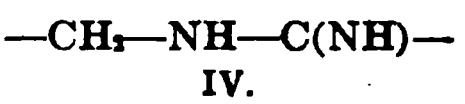


I.

II.



III.



IV.

In the synthetic polypeptides it may be inferred from the methods of synthesis employed that the amino-acids are united with one another through a nitrogen atom, and the fact that these polypeptides yield amino-acids on treatment with the same hydrolyzing agents that produce amino-acids from proteins, and the fact that polypeptides in which this structure has been established by synthesis, occur among the partial digestion-products of protein, combine to establish the correctness of the view that in the proteins, as in the synthetic polypeptides, the union of amino-acids takes place through the neutralization of amino-groups by carboxyl-groups. Furthermore the fact that the proteins yield the **Biuret-reaction** also confirms this view of the construction of the protein molecule. It has been found that only those substances which contain two —COHN— groups or two —CSHN— or two —C(NH)HN— groups, or under certain conditions, two —CHNH— groups yield the biuret-test. That such groups as

$$\text{—COHN—}\underset{|}{\overset{|}{\text{C}}}\text{—COHN—}$$

occur frequently in protein molecules, as they do in the molecules of the polypeptides, is therefore, established by a number of independent lines of evidence, and the existence of a large number of such linkages in the proteins is furthermore confirmed by the extreme paucity of **Free Amino-groups** in the native protein molecule.

The content of free amino-groups in native and derived proteins may be estimated in either of two ways. The first depends upon the liberation of nitrogen which occurs when free $—NH_2$ groups react with nitrous acid, in accordance with the equation:

$$RNH_2 + HNO_2 = ROH + H_2O + N_2$$

one molecule of nitrogen being released for every free amino-group originally present in the substance. The second method (Sörensen's method of formol-titration) depends upon the fact that formaldehyde reacts with amino-acids to form methylene derivatives in accordance with the reaction:

$$HOOCRNH_2 + HCOH = HOOCRN{:}CH_2 + H_2O.$$

If the solution be neutral to begin with, it will now be acid, owing to the destruction of the basic $—NH_2$-group leaving the carboxyl-group unopposed. The number of amino-groups destroyed by the formaldehyde, or rather the number of carboxyl-groups left unopposed, may be estimated by the quantity of alkali required to restore the original neutral reaction of the solution.

An examination of the various proteins by either of these methods reveals the fact that the content of free amino-groups in the unhydro-

lyzed protein molecules is very small indeed. Thus Van Slyke and Birchard have obtained the following results:

PERCENTAGE OF TOTAL NITROGEN PRESENT IN FREE AMINO GROUPS.

Hemoglobin	6.0
Casein	5.5
Hemocyanin	4.3
Gelatin	3.1
Edestin	1.8
Gliadin	1.1
Zein	0.0
Heteroalbumose	8.1
Protoalbumose	9.9

On the other hand, **Edestin**, after complete hydrolysis by hydrochloric acid, yields a volume of free nitrogen, on treatment with nitrous acid, corresponding to no less than seventy-nine per cent. of its total nitrogen content, and the formol-titration is proportionately increased. A very small proportion of the amino-groups of the amino-acids from which these proteins are built up are, therefore, present as unneutralized amino-groups in the unhydrolyzed protein.

It has, in fact, been shown that the free amino-nitrogen in the unaltered protein molecule exactly corresponds in quantity to one-half the **Lysine** nitrogen. Hence **Zein**, which contains no lysine radicals, yields no free nitrogen on treatment with nitrous acid. The period required for the complete interaction of proteins with nitrous acid is about thirty minutes (with the technique employed by Van Slyke) which is ten times as long as the period required for complete interaction with α-amino-groups, but corresponds exactly with that found to be required for the complete interaction of nitrous acid with the ω-amino-group of lysine. From these facts Van Slyke and Birchard infer that lysine is united to the adjacent amino-acid radicals in the protein molecule by means of its carboxyl- and its α-amino-groups, while the ω-amino-group remains uncombined and represents, within at most, a fraction of a per cent. of the total protein nitrogen, the entire amount of free amino-nitrogen in the native proteins. The α-amino-groups, which constitute by far the greater part of the free amino-nitrogen formed after complete hydrolysis are, in the intact protein molecule, all condensed into peptide-linkages.

In the primary **Proteoses**, or first split-products of protein hydrolysis, the relations are different. The free amino-groups in hetero- or proto-albumose exceed one-half the content of lysine nitrogen by 3.0 and 4.8 per cent. of the total nitrogen respectively, indicating that an appreciable proportion of the α-amino-groups are uncovered even in the first steps of hydrolysis.

Notwithstanding the fact, however, that a great majority of the linkages uniting the different amino-acids in the protein molecule have thus been proved to be of the peptide character, the possibility is by

no means excluded that other linkages may coexist with these in the protein molecule. One fact which would appear to afford indication of the presence of non-peptide linkages in the native protein molecule is that while native protein is attacked by the **Pepsin** of gastric juice and hydrolyzed by it as far as the proteose stage of hydrolysis, the various peptones and synthetic polypeptides are not hydrolyzable by pepsin. This may mean that types of union exist in the protein molecule which are susceptible to attack by pepsin and which are not present in the peptones and polypeptides. On the other hand it may simply indicate that the greater length of the amino-acid chain in the native protein molecule confers upon it sufficient instability to lay it open to attack and disintegration by the relatively weak hydrolyzing enzyme, pepsin, while the relatively stable proteoses require a more energetic hydrolyzing-agent such as the **Trypsin** of pancreatic juice. This latter view receives substantial support from the fact that the splitting of protein into proteoses already results in the uncovering of free amino-groups; so that the proteoses are evidently united together in the protein molecule through nitrogen atoms as the amino-acids are linked together in peptides. Furthermore, it is definitely known that greater length of an amino-acid chain confers upon it greater instability toward hydrolyzing enzymes. Thus, **Tetra-glycyl-glycine** is hydrolyzed by trypsin, while glycyl-glycin, diglycyl-glycine and triglycyl-glycine are not attacked by this enzyme.

CONSEQUENCES OF THE POLYPEPTIDE STRUCTURE OF PROTEINS.

The polypeptides are as essentially amino-acids as the amino-acids out of which they are built up. Thus, glycyl-glycine is as typically an amino-acid as glycine itself, since it possesses an $—NH_2$ group as well as a —COOH group, and for this reason is presumably capable of forming compounds, not only with acids and bases, but also, possibly, even with neutral salts, by attaching the basic radical of the salt at one point of the molecule (the carboxyl) and the acid radical at another (the amino-group). On undergoing electrolytic dissociation in aqueous solution it may be supposed to yield either hydrogen (H^+) ions, or hydroxyl (OH^-) ions, owing to the occurrence of a reaction with water of the type:

$$R\begin{cases}NH_2\\COOH\end{cases} + H.OH = R\begin{cases}NH_3OH\\COOH\end{cases}$$

just as ammonia, in aqueous solution, partially reacts with water to form NH_4OH.

It was considered until quite recently, and is still thought in some quarters, that these elements in the structure of the protein and polypeptide molecules afford an explanation of the power which they possess of uniting with both acids and bases, in other words the

Amphoteric character of the proteins. A variety of facts have been ascertained in recent years, however, which have compelled a revision of this opinion, and we now recognize that some elements in the protein molecule other than free $—NH_2$ or COOH groups must be responsible for the acid- and base-neutralizing power that is possessed in a marked degree by many proteins.

In the first place, the investigations to which reference has been made above have shown that only a very small proportion of the nitrogen in proteins is present within their molecules in the form of $—NH_2$ groups. Thus, in the case of **Edestin**, as the above-quoted estimations show, only 1.8 per cent. of the total nitrogen is present in the form of $—NH_2$ groups. Now edestin is insoluble, when in the free condition, in water. It forms an insoluble hydrochloride containing 14×10^{-5} equivalents[1] of hydrochloric acid per gram. On further addition of acid, soluble hydrochlorides are formed, and the substance passes completely into solution when the proportion of combined acid is just double that contained in the insoluble hydrochloride. Still further additions of acid, however, continue to be neutralized by the protein until, at neutrality to tropaeolin OO, which changes color when the amount of free acid in solution is between one-hundredth and one-thousandth normal, after due allowance for the acid which remains unneutralized, it is found that edestin combines with no less than 127×10^{-5} equivalents of acid per gram. The formation of the insoluble compound with 14×10^{-5} equivalents of acid must correspond to the union of at least one molecule of the acid with each molecule of protein, for any acid in excess of this amount results in the formation of a compound of quite a different character, namely, one which is soluble in water. The maximal number of molecules of acid which may be neutralized by a molecule of edestin has not been determined, but it evidently cannot be less than the number corresponding to 127×10^{-5} equivalents of hydrochloric acid per gram of protein. If we assume, therefore, that the insoluble compound represented the result of union of one molecule of acid with each molecule of protein, then the compound formed at neutrality to tropaeolin must represent the formation of a compound containing $\frac{127}{14}=9$ molecules of acid for each molecule of edestin. If this compound were formed by the union of the acid with $—NH_2$ groups in accordance with a series of reactions of the type:

$$RNH_2OH + HCl = RNH_2HCl + H_2O$$

then we would obviously have to assume the existence of no fewer than nine free $—NH_2$ groups in the molecule of edestin. If we assume the insoluble compound to have been formed by the union of *two* molecules of acid with one of edestin, then the estimate of the number of free $—NH_2$ groups must be raised to $2\times9=18$ and so forth.

[1] That is, the combining weight of HCl expressed in grams and multipled by 0.00014, or the hydrochloric acid which is present in 1.4 c.c. of a tenth normal solution.

Supposing the insoluble compound to have been formed by the union of only one molecule of acid with one molecule of edestin, since 1 gram of the protein is, in this compound, equivalent to only 14×10^{-5} molecule of hydrochloric acid, about 7000 grams of protein would be neutralized by one gram-molecule of hydrochloric acid. The molecular weight of edestin, if the insoluble compound is formed by the union of one molecule of hydrochloric acid with one molecule of protein, must therefore, be about 7000. Nine $—NH_2$ groups in this molecule would correspond to ten per cent. of the total which edestin contains. If we were to assume that the insoluble compound contains two molecules of hydrochloric acid per molecule of protein, then our estimate of the molecular weight of edestin would have to be doubled, but as the estimated number of free $—NH_2$ groups would also be doubled, this would leave us still the same proportion, ten per cent., of the total nitrogen in the molecule in the form of free $—NH_2$ groups.

Now the measurements of Van Slyke and Birchard have shown that only 1.8 per cent. of the total nitrogen of the edestin molecule is present therein, in the form of free $—NH_2$, groups, so that no less than 8.2 tenths or eighty-two per cent. of the neutralizing-power of edestin for acids remains to be accounted for in some other fashion than by the assumption of a union of the acid with free $—NH_2$ groups.

The same or similar measurements have been made, employing a diversity of proteins, and always with the same disparity between the actual proportion of free amino-groups in the molecule of the protein and the proportion which would be required to accomplish the neutralization of all the acid which the protein is capable of binding. Thus, it has been pointed out by Kossel and Cameron that the acid-combining capacity of the protamine, **Salmine** is equal to the combining-capacity of all of the guanidine-groups of the arginine radicals which this protein contains. Yet salmine yields no nitrogen whatever on treatment with nitrous acid. **Sturine**, which is another protamine, contains 67 per cent. of its nitrogen in the form of histidine and 6 to 7 per cent. in the form of lysine. It yields nitrogen on treatment with nitrous acid corresponding to the ω-amino-group of the lysine. Only about three or four out of every hundred nitrogen atoms in sturine are therefore present in the form of free amino-groups. Yet one hundred nitrogen atoms in sturine will neutralize no less than twenty-four equivalent gram-molecules of acid. Evidently at least twenty of these acid molecules must attach themselves to the molecule of protein at some other points than those provided by free $—NH_2$-groups.

The number of **Free Carboxyl-groups** in any protein cannot be much in excess of the number of free amino-groups, for otherwise the protein would be overwhelmingly acid in its character, and behavior, and besides, since relatively few of the amino-acid radicals in most proteins are dicarboxylic acid radicals, if a great excess of free carboxyl-groups were present in the molecule, the combined amino-groups could not all be attached to carboxyl-groups as they are in the polypeptides, and

as all our evidence tends to show they are in the proteins. Moreover the results of the formol-titration show that there are not many free carboxyl-groups in the protein molecule, and the same conclusion may be reached from a consideration of the effects of rather concentrated alkali in bringing about "racemization" or optical inactivity of the majority of the amino-acid radicals in proteins. It is found that amino-acid radicals of which the carboyxl-group remains uncombined, are not "racemized" by alkali, while amino-acids of which the carboxyl-groups are neutralized in peptide-linkages are rendered optically inactive by strong alkalies. The great majority of the amino-acids which result from the alkaline hydrolysis of proteins are optically inactive and so we must assume that in the native protein molecule their carboxyl-groups were not unattached.

Now uncombined **Casein** is insoluble in water, but when combined with acids or with bases it is soluble. When just sufficient alkali has been employed to carry every particle of casein into solution at least one molecule of the alkali must have combined with each molecule of casein. To carry one gram of casein into solution 11.4×10^{-5} equivalents of base, or 1.14 c.c. of tenth normal alkali just suffice, indicating a combining-weight for casein of about 8800. The tyrosin and sulphur-contents of casein indicate that the molecular weight of casein must be some multiple of 4400.

In the presence of *excess* of alkali, however, the combining capacity of casein for bases is very much greater. We cannot, of course, determine the maximal combining-capacity of casein for bases by titration, because the removal of the uncombined excess of alkali by the acid used in titration simply results in reducing the combining-capacity of the casein for the alkali, just as the running in of acid into a solution of sodium carbonate results in the formation of sodium bicarbonate. Nor is it convenient to determine the maximal combining-capacity of proteins for bases by means of indicators. The method employed is to determine the quantity of uncombined alkali in the protein solution electrometrically by means of the **Gas-chain** (see Chapter XII), that is by the potential developed at the surface-layer of an electrode of hydrogen dipped into the protein solution. The greater the concentration of free alkali, *i. e.*, of hydroxyl ions in the solution the less, in proportion, must be the concentration of free hydrogen ions, and the less the concentration of free hydrogen ions in the solution the more hydrogen ions will travel from the superficial layer of the electrode into the superficial layer of solution which is in contact with it. These hydrogen ions carry with them a positive charge, and hence the solution becomes charged positively and the electrode carries a corresponding negative charge. The potential thus created is a measure of the alkalinity (or acidity) of the solution under investigation. For the **Hydrogen Electrode** we use a piece of platinum foil or platinum gauze coated with platinum-black and saturated with hydrogen gas.

By this method it may be shown that in the presence of an excess of alkali, casein combines with a *maximal* proportion of 180×10^{-5} equivalents of base per gram. The combining-capacity of casein for alkalies does not exceed this figure no matter what excess of alkali we may employ. We have seen that the *minimal* combining-capacity of casein for bases is 11.4 equivalents of base per gram. Hence, reasoning as we did in the case of the compounds of edestin with hydrochloric acid, if the minimal proportion of alkali which just suffices to carry casein into solution corresponds to the union of one molecule of alkali with one molecule of casein, the maximal proportion of alkali which may be bound by casein must correspond to the union of at least sixteen molecules of base with one molecule of protein. If these molecules of alkali were united to the protein through —COOH-groups there must be sixteen of them, or over one-fourth of all of the carboxyl-groups in the protein must exist therein in the free, uncombined condition. We have seen that this would be impossible excepting in the case of the second carboxyl in the dicarboxylic acid radicals and of these there are only sufficient in casein to supply one-half of the carboxyl-groups required. Evidently the union of bases with casein is accomplished through some agency other than free carboxyl-groups.

The nature of the radicals which accomplish the union of protein with alkalies is indicated by the experiments of H. M. Vernon, who has compared the power of proteins and of their hydrolytic decomposition products to neutralize bases. Although the **Hydrolytic Decomposition-products** of a protein will neutralize more alkali than the undecomposed protein, yet the gain in power to neutralize bases is very much less than one would anticipate in view of the large number of carboxyl-groups which are set free by hydrolysis. In fact the alkali-neutralizing power of the hydrolyzed protein is only slightly greater than the alkali-neutralizing power of the native, undecomposed protein. Now in the process of hydrolysis the —COHN— groups of the protein are split into —NH_2 and —COOH groups. The inference is that the —COHN— -groups within the protein molecule must be nearly as efficient in accomplishing the neutralization of bases as the —COOH groups of the constituent amino-acids out of which the protein is built up.

More direct evidence that the —COHN— groups in the protein molecule are responsible for the neutralization of bases by proteins is afforded by the investigations of Osborne and Leavenworth, who have shown that **Edestin**, for example, combines with and holds in solution 34.67 per cent. of its weight of copper in the form of the otherwise insoluble cupric hydroxide. This, if we assume that each copper atom unites with one nitrogen atom, involves the union of cupric hydroxide with ten out of every sixteen atoms of nitrogen in the edestin molecule. Now this is exactly the proportion of nitrogen which edestin yields in the form of amino-nitrogen after complete hydrolysis. In other words,

it is exactly equal to the proportion of —COHN— -groups which the unhydrolyzed molecule contains; precisely similar results were obtained with **Gliadin.**

Direct proof on the other hand, that free —NH_2-groups are not responsible for any appreciable proportion of the acid-combining capacity of proteins has been furnished by the experiments of Blasel and Matula, and of Pauli and Hirschfeld. These investigators prepared **Deaminized Gelatin** by acting upon gelatin with nitrous acid, thus destroying all the free —NH_2-groups in the molecule. They then compared, with the aid of the hydrogen electrode, the acid-combining capacity of the deaminized gelatin with that of normal gelatin. They found that the combining-capacity of deaminized gelatin for acids is only very slightly inferior to that of normal gelatin, indicating, beyond any question, that the combining-capacity of gelatin for acids is, in very large proportion, attributable to elements of the molecule other than free —NH_2-groups. Since nitrogen atoms must certainly be the agents through which union of acids with protein is brought about, the inference is unavoidable that the elements of the molecule which actually accomplish the binding of acids by protein are, in very large proportion the —COHN— -groups within the body of the protein molecule.

To account for both the acid- and the base-combining capacity of the proteins we must therefore look, not to the small proportion of free —NH_2 or —COOH-groups which the proteins afford, but to the —COHN— -groups within the body of the molecule. Now two varieties of this linkage can be conceived, between which it has not proved possible as yet, to decide by any direct method of analysis. Thus **Glycyl-glycine** may conceivably be either:

Keto-Form.

$$H_2N.CH_2.CO—HN.CH_2.COOH$$

or:

Enol-Form.

$$H_2N.CH_2.C(OH)=N.CH_2.COOH$$

and our analytical data, and the modes of decomposition and synthesis of the proteins and peptides do not enable us, with any degree of certainty, to distinguish between them. Neither form is therefore inconsistent with our present knowledge of the synthesis and hydrolysis of proteins and polypeptides, but while the keto-form of the —COHN— -group would conceivably possess the power of neutralizing acids, it offers no probable point of union for bases. The enol-form, on the contrary, would provide a point of union for either acids or bases.

According to Werner's theory of valencies, the nitrogen in either of these types of linkage contains two latent valencies, positive and negative, which, while the nitrogen remains trivalent, neutralize one another, but when the nitrogen becomes pentavalent are capable, respectively, of neutralizing a negative and a positive radical. The

enol type of union carries with it the possibility of the following types of reaction:

$$—COH{=}N— + Na^{+} + OH^{-} = (—CONa)^{++} + \left(\begin{array}{c} H \\ | \\ N— \\ | \\ OH \end{array}\right)^{--}.$$

and

$$—COH{=}N— + H^{+} + Cl^{-} = (—COH)^{++} + \left(\begin{array}{c} H \\ | \\ N— \\ | \\ Cl \end{array}\right)^{--}$$

yielding, in each case *only protein ions*.

This conception of the mode of union of proteins with inorganic acids and bases affords an explanation of what would otherwise constitute a very puzzling fact, namely, that while compounds of acids or bases with protein form very good conductors of electricity through their solutions, and must, therefore, be electrolytically dissociated into ions, yet no evidence is afforded by these solutions of the existence in them of any ions derived from the combined acid or base. Thus chlorides of the proteins yield only traces of chlorine ions in solution, as is shown by the difficulty or sluggishness with which they react with silver salts to form silver chloride. The calcium compound of **Casein** does not dissociate any appreciable proportion of calcium ions, and compound of the proteins with silver, mercury, lead, copper, etc., do not yield up these ions to the solution. The conductance of electricity through the solution of a protein compound cannot be due to contamination of the protein with dissociable inorganic salts, because the conductivities observed are too large to be accounted for in this way and, moreover, the conductivity of the protein compound varies in a very striking and regular manner with the proportion of acid or alkali bound, increasing, as one might expect, with the number of acid or alkali radicals which have entered into combination with the protein. Furthermore, in many instances, *e. g.*, **Casein** and **Serum-globulin**, it is easy to show that the protein participates in the conductance of the current owing to the fact that the protein is precipitated at one of the electrodes, and the amount of protein so precipitated is proportional to the amount of current which has traversed the solution. It is a very striking fact that casein in alkaline solution, although it is only precipitated at the one electrode (the anode) yet migrates toward both electrodes when a current is passed through the solution, indicating that certain portions of the casein are engaged in transporting positive, while others are transporting negative charges. Migration of the proteins toward both electrodes has also been observed in solutions of **Hemoglobin** and in solutions of **Fibrinogen.**

THE PRECIPITATION AND COAGULATION OF PROTEINS BY INORGANIC SALTS.

The precipitation of proteins, and, indeed, of **Colloids** in general, may be of two kinds: The first is clearly accompanied by decomposition of the precipitating agent, it will not occur unless the protein is ionized, *i. e.*, migrates under the influence of an electric current; and only small quantities of the precipitating-agent are required to bring about the precipitation. The second kind of precipitation, however, whether accompanied by decomposition of the precipitating-agent or not, occurs even when the protein is non-ionized, and requires relatively large amounts of the precipitating-agent. Precipitation of the first kind is, generally speaking, only brought about by electrolytes, while precipitation of the second kind, although as a rule, more readily brought about by electrolytes than by non-electrolytes, may nevertheless be brought about by certain non-electrolytes, for example, by alcohol. For this latter type of precipitation we shall henceforth reserve the term **Coagulation.**

Both **Precipitation** and **Coagulation** of a protein may be brought about by one and the same inorganic salt. In such a case the gradual addition of salt to the originally salt-free solution which contains ionic protein, *i. e.*, protein which drifts to one electrode or to the other in an electric field, first brings about precipitation and then resolution of the protein. In this new solution the protein appears to be invariably *non-ionic*, and it can be coagulated by still further addition of the salt.

The first kind of precipitation appears to be undoubtedly chemical in character and in mechanism. The mechanism of coagulation is, however, far from clear, and for the attainment of an adequate understanding of this phenomenon we shall doubtless have to wait until the physicochemical theory of phenomena of solution in general has reached a more mature stage of development than it has at present. There would appear to be no room for doubt, however, that processes of **Dehydration** play an important and perhaps a decisive part in bringing about coagulation.

The concentrations of the various inorganic salts which are required to bring about the precipitation of a colloid in solution very greatly depend upon the **Electrical Sign** of the inorganic ions with which it may chance to be combined, and upon the **Valencies** of the ions of the salts used for precipitating the colloid. Thus, defining the "**Precipitating-power**" of a salt as the reciprocal of the concentration, in gram-molecules per liter, necessary to precipitate a given solution of colloidal **Sulphide of Arsenic**, Schultz found that the relative precipitating-powers of the univalent, divalent and trivalent metals are in the ratios 1 : 30 : 1650. Similar figures were obtained for colloidal **Cadmium Sulphide**, while Linder and Picton, using colloidal **Antimony Sulphide**, found that the precipitating-powers of different salts of a given metal are proportional to their equivalent conductivities, *i. e.*, to the concentration of metal

ions present in the mixture, and that the relative precipitating-powers of the sulphates of univalent, divalent and trivalent metals can be expressed by the ratios 1: 35 : 1923.

In all of these cases the colloid employed was **Electronegative**; that is, in electrolysis it was precipitated at the anode, the colloid behaving like the acid radical of a salt. The experiments which we have cited show that in such instances the ion of the added electrolyte which is effective in bringing about precipitation is the cation, since the valency of the cation determined the precipitating-power of the salt, while the valency of the anion was immaterial to the result. If, however, we dilute the white of an egg with ten times its volume of distilled water, filter off the flakes of **Globulin** which are precipitated, and then heat the solution to boiling-point, we obtain an opalescent solution or suspension of **Egg-albumin** which is very easily coagulated by traces of various salts. If a trace of acid be added to the solution and an electrical current passed through it, the protein is precipitated at the cathode, while if, instead of acid, a trace of alkali be added, the protein is precipitated, not at the cathode but at the anode. In the former case the protein behaves like a cation or the basic radical of a salt, and is said to be "**Electropositive**," while in the latter case the protein behaves like an anion, or the acid radical of a salt, and it is said to be "**Electronegative**." It is probable that in both these instances the protein migrates to both electrodes, but the protein ion which carries the inorganic radical with it, in the first case the acid and in the second the alkali, is held in solution by the acid or alkali it bears after it has given up its electrical charge to the electrode.

Now in alkaline solutions of this heat-modified egg-albumin it is found that the *cations* of added salts are the active agents in precipitating the protein, just as in the case of the sulphides of antimony, arsenic or cadmium, but in acid solutions of the heat-modified egg-albumin these relationships are entirely reversed, the valency of the cation of the added salt becomes immaterial and the precipitating-power of the salt is determined by its *anion*. The following results, obtained by W. B. Hardy, illustrate this inversion of the precipitating ion when the protein, from functioning as an acid, comes to function as a base:

PROTEIN IN PRESENCE OF A TRACE OF ALKALI; ELECTRONEGATIVE.

Temperature 16 degrees. Coagulating salt 1 gram-mol. in 80.000 c.c.

Coagulated at once.	On slightly warming.	Did not coagulate.
$Al_2(SO_4)_3$	$MgSO_4$	Na_2SO_4
$Cd(NO_3)_2$	$BaCl_2$	K_2SO_4
$CuSO_4$	$CaCl_2$	NaCl
$CuCl_2$		

PROTEIN IN PRESENCE OF A TRACE OF ACID; ELECTROPOSITIVE.

Coagulated instantly.	No effect.
$Al_2(SO_4)_3$	$CuCl_2$
$CaSO_4$	$Cd(NO_3)_2$
K_2SO_4	$BaCl_2$
Na_2SO_4	NaCl
$MgSO_4$	

Similar results have been obtained with a variety of other colloids, Electronegative colloids are precipitated, if at all, by cations; electropositive colloids by anions.

Whetham explained these phenomena in the following way: He assumes that each colloidal particle carries an electrical charge, a corresponding and opposite charge being induced upon the surface of the water in immediate contact with the colloid. The effect of this charge is to diminish the **Surface-tension** at the surface separating the water and the colloid, and therefore, to diminish the tendency of this surface to contract. So long as the colloid is dispersed through the solution in the form of minute suspended or dissolved particles the surface separating the colloid and the water is very large. When the colloid is flocculated and precipitated the surface is, in consequence, contracted. The less the tendency of this surface to contract, Whetham argues, the less will be the tendency for the colloidal particles to adhere to one another and form large flocculi.

The cations of the added electrolyte, in the case of "electronegative" colloids, or the anions of the added electrolyte, in the case of "electropositive" colloids, neutralize, according to Whetham, the charges which are carried by the colloidal particles. The electrical double layer at the surface of the colloid and the water thus disappears, and the surface contracts; the finely suspended colloidal particles unite to form large aggregates having a less extended surface and these aggregates finally become so large as to assume the properties of matter in mass, and hence are carried out of solution by the action of gravity. In this way the dependence of the precipitating-power of an electrolyte upon its degree of ionization, and also the reversal in the relative precipitating-powers of the ions of the added electrolyte upon reversion of the sign of the electrical charge presumed to be carried by the colloid, found an explanation. In interpreting the **Valency Rule** discovered by Schultz, Whetham develops his theory as follows:

"In a solution where ions are moving freely, the probability that an ion is at any instant within reach of a fixed point is, putting certainty equal to unity, approximately represented by a fraction proportional to the ratio between the volume occupied by the spheres of influence of the ions and the whole volume of the solution and may be written as A C, where A is constant and C represents the concentration of the solution. The chance that two such ions should be present together is the product of their separate chances, that is $(AC)^2$. Similarly the chance for the conjunction of three ions is $(AC)^3$, and for the conjunction of n ions is $(AC)^n$."

"In order that three solutions containing trivalent, divalent and univalent ions respectively should have equal coagulative powers, the frequency with which the necessary conjunctions should occur must be the same in each solution. We should then have, the constant being assumed equal in each case:

$$A^{2n}C_3^{2n} = A^{3n}C_2^{3n} = A^{6n}C_1^{6n} = \text{a constant} = B$$

Therefore

$$C_3 = \frac{B^{\frac{1}{2n}}}{A}; \qquad C_2 = \frac{B^{\frac{1}{3n}}}{A}; \qquad C_1 = \frac{B^{\frac{1}{6n}}}{A}$$

C_1, C_2, C_3 representing the concentrations of monads, diads and triads in their respective solutions. Thus we get for the ratios of the concentrations of equicoagulative solutions:

$$C_1 : C_2 : C_3 = B^{\frac{1}{6n}} : B^{\frac{1}{3n}} : B^{\frac{1}{2n}} = 1 : B^{\frac{1}{6n}} : B^{\frac{1}{3n}}$$

Let us put $B^{\frac{1}{6n}} = \frac{1}{x}$; the ratios can then be written:

$$1 : \frac{1}{x} : \frac{1}{x^2}$$

The reciprocals of the numbers expressing the relative concentrations of equicoagulative solutions give values proportional to the coagulative powers of solutions of equal concentration; so that, calling the coagulative-powers of equivalent solutions containing monovalent, divalent and trivalent ions respectively $p_1 : p_2 : p_3$, we get:

$$p_1 : p_2 : p_2 : = 1 : x : x^2$$

Let us now take some numerical examples;
Putting $x=32$ we get the series:

$$1 : 32 : 1024$$

which agrees very well with Linder and Picton's results for colloidal solutions of antimony sulphide:

$$1 : 35 : 1023$$

and putting $x=40$, we get

$$1 : 40 : 1600$$

numbers comparable to Schultze's values for sulphide of arsenic.

This theory obtained for some time a very wide acceptance, but the difficulty attaching to it from the first was that of accounting for the acquirement, by the colloid, of an electrical charge. It will be recollected that the sign of the charge borne by the portion of the colloid which is precipitated at an electrode by an electric current, is reversed by changing the reaction of the solution of the colloid from acid to alkaline. Obviously, therefore, the electrical charge carried by the colloidal particles is determined by the acid or alkali which is added to the solution, and it was tacitly assumed that the charge obtaining in acid solutions was derived from the hydrogen ions of the acid, while that obtaining in alkaline solutions was derived from the hydroxyl ions of the alkali. But if, for example, egg-albumin acquires

a positive charge from the hydrogen ions of hydrochloric acid, we must next inquire what becomes of the chlorine ion of the acid? It cannot exist in a free state with unneutralized charges, and no way exists for it to neutralize its charge except by attachment to an oppositely charged albumin molecule. But this attachment of both ions of the hydrochloric acid to the albumin differs in no distinguishable way whatever from chemical combination.

That the hydrogen ions of acids are actually bound by the protein may readily be shown by employing the hydrogen electrode or even by the aid of indicators. The anions of acids must therefore be likewise attached to the protein particles. Now let us suppose that the charge communicated to the protein particle by the hydrogen ion of the acid is neutralized by the anion of a precipitating salt, what will happen to the cation of the precipitating salt? Its charge must be neutralized, and this can only be accomplished by its union with the protein complex or else by its union with the acid radical which is also attached to the protein. In the first alternative, the train of events would be represented by the equation:

$$2\,\text{Protein HCl} + Na_2SO_4 = (\text{Protein HCl})_2Na_2SO_4$$

and in the second alternative by the equation:

$$2\,\text{Protein HCl} + Na_2SO_4 = \text{Protein}_2H_2SO_4 + 2NaCl$$

In other words, from whatever point of view we may regard the precipitation of proteins by inorganic salts, as soon as we examine closely the details of the process, it becomes indistinguishable, by any criterion which we at present possess, from a chemical reaction, and it seems to be quite unnecessary to invent a special hypothesis to account for this particular type of chemical reaction, the need of which is not experienced in interpreting any other of the immense variety of chemical reactions yielding precipitates with which we are familiar.

It remains, however, to account for the peculiar relationship of precipitating power to the valency of the precipitating ion which led to the elaboration of Whetham's hypothesis. Now this hypothesis, when carefully examined, is seen to consist in nothing more than a restatement, in terms of the probabilities of molecular collisions, of the **Guldberg and Waage Mass-law** which applies to all chemical reactions. According to this law, the *velocity* with which any given chemical reaction proceeds, varies directly as the active masses of each of the reacting molecules. In the case under consideration, presuming that a given number (*e. g.* one) of molecules of protein react with one molecule of a salt of a monovalent metal to form a compound, then twice as many molecules of the protein may be supposed to react with a molecule of a salt of a divalent metal, and three times as many with a salt of a trivalent metal. Assuming that the active mass of the colloid (the molecular concentration multiplied by the degree of electrolytic dissociation)

is the same in each of these cases (which is also assumed in Whetham's theory) and equal to A, calling the *initial velocities* of the respective reactions v_1, v_2 and v_3 and the concentrations of the mono- di- and trivalent ions c_1, c_2 and c_3 we have:

$$v_1 \text{ is proportional to } A^1c_1$$
$$v_2 \text{ is proportional to } A^2c_2$$
$$v_3 \text{ is proportional to } A^3c_3$$

whence it follows that if $v_1 = v_2 = v_3$ and the velocity-constants of the three reactions are equal (which is also assumed in Whetham's hypothesis):

$$\frac{C_1}{C_1} : \frac{C_1}{C_2} : \frac{C_1}{C_3} = 1 : A : A^2$$

and

$$\frac{1}{C_1'} \quad \frac{1}{C_2} \quad \text{and} \quad \frac{1}{C_3'},$$

i. e., the dilutions of the mono- di- and trivalent ions at which combination proceeds with equal velocity, are related to one another in the same way. Now in the experiments described above, $\frac{1}{c}$ is defined as p_1 the precipitating-power of the salt, hence:

$$p_1 : p_2 : p_3 = 1 : A : A^2$$

which is exactly the relation deduced by Whetham. The experimental relations found by Schultz, Linder and Picton, Hardy and others are, therefore, just as explicable upon the assumption that the colloid reacts chemically with the precipitating ion as upon the assumption that the precipitating ion acts in a purely physical way through altering the electrical condition of the colloidal particles. The former view attributes to the colloids in general, and to the proteins in particular no especial qualities which differentiate them from other chemical systems, while the latter view necessitates radical assumptions regarding the nature of colloidal solutions which have hitherto proved incapable of verification.

It will be noticed, however, that the factor that determines the precipitating-power of a salt is the *velocity* with which it combines, with the protein and not the final *equilibrium* which is attained. This is not surprising when we recollect, firstly, the enormous part played by the *velocity of change* in determining the final physical condition of a colloid, and secondly, the method by which the "precipitating-powers" of salts are measured. Linder and Picton, for example, measured the precipitating-power of salt solutions by titration, running the solution of the salt into the solution of the colloid until precipitation just began to be perceived. They expressly state that unless the time occupied in the titration be kept approximately the same, serious deviations from the "valency rule" occur: "As a quantity of coagulant insufficient to produce coagulation immediately, will do so in the

course of time." Under these conditions, what is actually measured is the concentration of the precipitating-agent which is requisite to bring about a given degree of change (visible precipitation) within a given brief period, that is to say, a velocity, and not an equilibrium.

That protein, when it is precipitated by inorganic salts, actually enters into combination with them and carries down a portion of the precipitating salt, has been shown in a variety of instances. The most exhaustive investigations of this character have been those of Galeotti who has employed electrochemical methods of measuring the concentration of individual ions in the solutions. By these means he has been able to show, for example, that when **Egg-albumin** is precipitated by silver nitrate, Ag^+ and NO_3^- ions are removed from the solution and precipitated with the protein in equivalent proportions; in other words the protein combines with the whole molecule of silver nitrate to form an insoluble compound. It has also been shown that copper sulphate combines as a whole with egg-albumin to form an insoluble compound, but in alkaline solutions in the presence of an excess of the salt this precipitate redissolves and the albumin is now found to have combined with an excess of the copper, forming a *soluble* compound, while the alkali takes up the excess of sulphuric acid thus set free.

The very important observation has been made by Pauli that absolutely **Electrolyte-free Egg-albumin**, prepared by prolonged dialysis, is not ionic (*i. e.*, does not drift in an electric field) and that under these conditions it is not precipitable even by heavy metals. It is, however, *coagulated* by highly concentrated salts. In correspondence with this it has been shown by Rohmann and Hirschstein that the amount of silver nitrate which will combine with **Casein** to form an insoluble compound is exactly equivalent to the amount of base (NaOH) previously combined with the casein, *i. e.*, to the number of —COHN— -linkages that have been opened up and ionized by union with an inorganic base.

When we now turn from the phenomenon of **Precipitation** to that of **Coagulation** we meet with quite a different series of relationships. Instead of the **Coagulative-power** of salts being determined primarily by valency, we find that specific ions of varying valencies have high coagulative-powers, while others have low coagulative-powers, and these specific relationships are rather constant for a wide series of proteins and of other colloids. In coagulation, also, both ions of the coagulating-salt participate in determining coagulative-power, although they act in opposite senses, the cations coagulating and the anions inhibiting coagulation, or else *vice versa*. Thus, Pauli found that in egg-white (in which the protein is electronegative) the cations of added electrolytes are the active agents in inducing coagulation, while the anions inhibit coagulation. In the following table of Pauli's the cations are arranged in ascending order of precipitating-power from left to right, while the anions are arranged vertically, the weakest

inhibitor coming first and the strongest last. A (+) indicates that the salt which results from the union of the cation and anion causes coagulation of **Egg-albumin**; while a (−) indicates that it does not.

	Cations.				
Anions.	**Mg**	**NH**	**K**	**Na**	**Li**
Fluoride	....	+	+	+	....
Sulphate	+	+	+	+	+
Phosphate	....	+	+	+	....
Citrate	....	+	+	+	....
Tartrate	....	+	+	+	....
Acetate	−	−	+	+	....
Chloride	−	−	+	+	+
Nitrate	−	−	−	+	+
Chlorate	....	−	−	+	....
Bromide	−	−	−	−	+
Iodide	....	−	−	−	....
Thiocyanate. . . .	−	−	−	−	....

When the protein is combined with acid, however, or is "electropositive," the order of effectiveness of the different salts in bringing about coagulation is exactly the reverse of the order of effectiveness in bringing about the coagulation of protein combined with alkali ("electronegative") protein. The series is reversed in every respect; the anions now induce coagulation and the cations inhibit it. The anions coagulate in the order:

$$CNS > I > Br > NO_3 > Cl > CH_3COO$$

while the cations inhibit coagulation in the order:

$$Li > Na > K > NH_4 > Mg$$

We have seen that, in order that **Precipitation** of a protein by salts may occur, the protein must be ionized, but for **Coagulation** this condition is not requisite. In determining the rate of precipitation the valency of the precipitating ion is of prime importance; in determining the rate of coagulation it is of comparatively subordinate importance. For precipitation very low concentrations of the precipitating salt suffice, intermediate concentrations frequently, and indeed usually redissolve the precipitate, and for coagulation high concentrations of the salt are required. This latter fact, and the fact that the presence of coagulating-salts aids coagulation by alcohol and by heat, suggests, as it did to Hofmeister, that coagulation is dependent upon **Dehydration** of the protein.

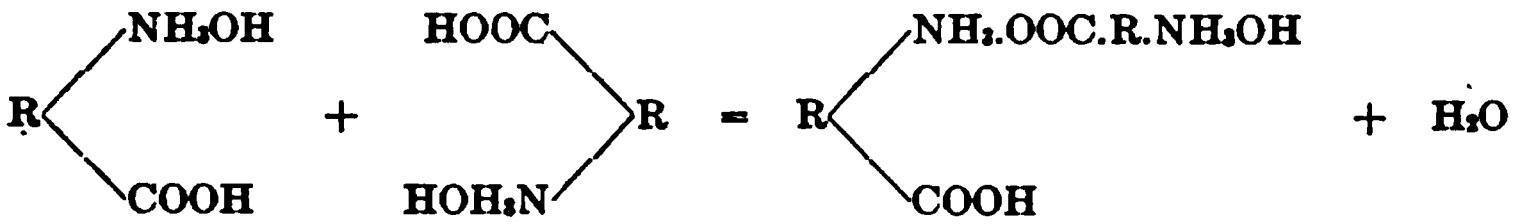
Starting from the observation of Jones and Ota, that certain salts when dissolved in water, produce an abnormal depression of the freezing-point, H. C. Jones and his pupils have built up a very large body of evidence for the existence of hydrates (or "solvates") of substances in solution. These investigators find that both ions and undis-

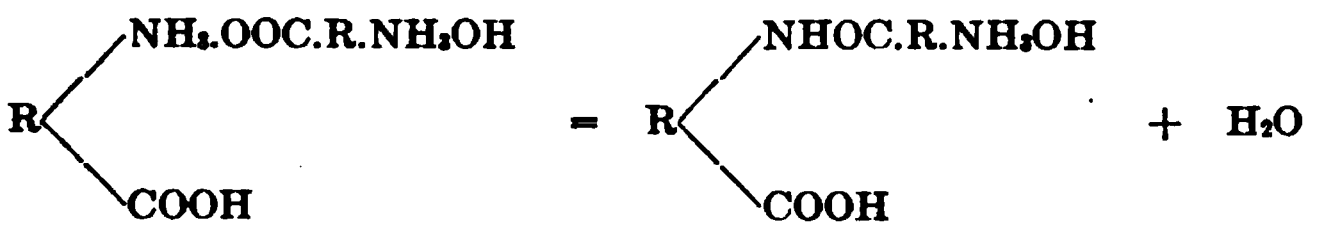
sociated molecules can form "solvates," and that these hydrates or "solvates" are readily decomposed at temperatures which approach the boiling-point of the solvent, and by the presence of other agents in the solution which compete for the solvent. The determination of the *quantity* of water bound in this way by substances in aqueous solution, is frequently a matter of difficulty and uncertainty, but the existence of such "solvate" compounds may be demonstrated in a variety of ways, although their quantitative composition remains, in general, unknown. A very striking experiment which illustrates the formation of "solvates" is that cited by Pickering. If a mixture of **Propyl Alcohol** and water be placed in a semipermeable vessel and surrounded with water, it is found that water enters the cell, but that no propyl alcohol escapes. If, however, the same semipermeable vessel, containing the same mixture of propyl alcohol and water, be immersed in propyl alcohol, propyl alcohol enters the cell and water does not leave it. In other words, the vessel is permeable to either propyl alcohol or water when these are pure, but it is impermeable to mixtures of the two, the inference being that large molecular complexes are formed on mixing these reagents which cannot pass through the pores of the vessel. From these and similar experiments Poynting concludes that osmotic pressure is an expression of the diminution in the active mass of the solvent due to the formation of compounds with the dissolved substance.

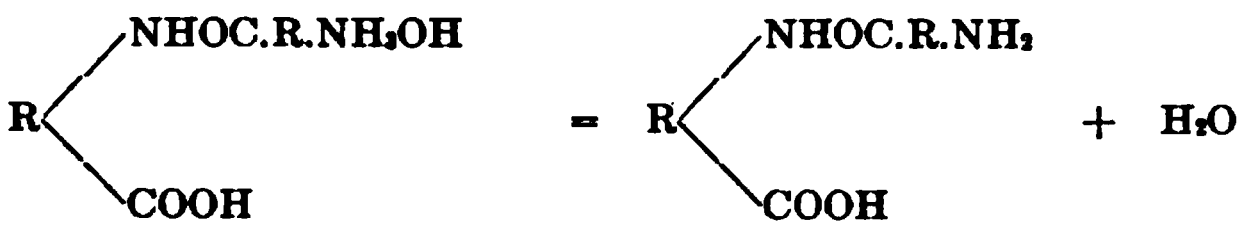
It is a familiar fact to chemists that anhydrous **Cobalt Chloride** is blue, but that on taking up water it becomes violet or red. Ostwald believed that the undissociated cobalt chloride molecule is blue, while the cobalt ion is red. Since, however, the color of a concentrated solution of cobalt chloride can be changed from purplish-red to blue by the addition of relatively small amounts of calcium salts, or still smaller amounts of aluminium chloride, or by the addition of a few drops of alcohol, it is more probable that this change in color is due to dehydration of the cobalt chloride molecule in solution, by the abstraction of water from it by the added substance. Similarly the progressive change in color of **Cupric Chloride** solutions, from blue to greenish-brown, on concentration or dehydration is attributed to the loss of water on the part of cupric chloride water-complexes. G. N. Lewis finds that if various bromides be added to concentrated solutions of **Cupric Bromide** the copper salt is dehydrated (turned brown) by the salts of monovalent metals in the order: $Li > Na > NH_4 > K$. Divalent metals dehydrate more strongly, the order being: $Mg > Ca > Sr > Ba$ while trivalent metals (Al) act still more energetically. The resemblance between the order of effectiveness of the monovalent metals in dehydrating cupric bromide and their order of effectiveness in coagulating "electronegative" protein is very evident.

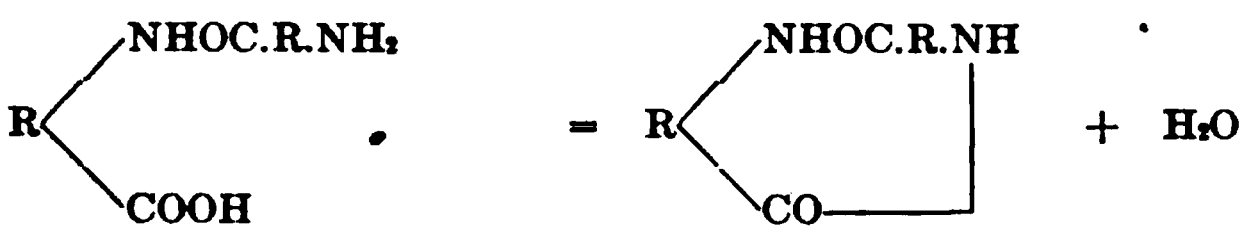
The peculiar interest to the biological chemist of the possibility thus indicated, that substances dissolved in water form loose combinations with the solvent, lies in the especial significance of water in relation to

the protein and polypeptide structure. Dehydration of a protein may result in one or more of the following series of reactions:









and hydration, of course, may result in the reversion of this series of changes.

That proteins may be thrown out of solution in two very different conditions of hydration is very clearly shown by the following experiments:

Anhydrous **Casein** dissolves readily in cold anhydrous **Formic Acid**; still more readily in hot formic acid. If, to a two per cent. solution of casein in formic acid, we add a fairly concentrated solution of **Cupric Chloride**, the mixture is at first green, indicating the presence of lower hydrates of cupric chloride, but on adding more of the solution it becomes blue, and simultaneously with the appearance of a pure blue color, but not before, precipitation of cupric caseinate occurs. If, to five c.c. of a two per cent. solution of casein in formic acid, we add 1½, 2 or 2½ c.c. of a saturated solution of cupric chloride, no precipitation of the caseinate occurs, but on diluting this mixture with water a precipitate results, and the appearance of this precipitate coincides with the attainment of a clear blue color on the part of the mixture.

About six cubic centimeters of water are required to produce a permanent precipitate. This precipitate redissolves on heating, and *the mixture simultaneously becomes green;* on cooling the blue color reappears and with it the precipitate. If formic acid be added to the mixture the precipitate redissolves as soon as the mixture becomes green. If the precipitate be very slight it will redissolve on adding alcohol. It cannot be urged that the formation of cupric caseinate requires the presence of more cupric ions than are present in green solutions, because green solutions of cupric chloride contain an abundance of ions, and casein will react with very small amounts of metal

ions, for although it is itself insoluble it will drive carbonic acid out of the sparingly soluble calcium carbonate to form a freely soluble caseinate of calcium.

If instead of adding *water* to a mixture of five c.c. of two per cent. casein in formic acid, and two c.c. of saturated cupric chloride, we add *alcohol;* no coagulation occurs until the mixture changes in color from green to brown, when a **Coagulum** of cupric caseinate is produced which redissolves on adding water.

Similar results are obtained when a 2-molecular solution of **Cobalt Chloride** is employed instead of a saturated solution of cupric chloride. If to five c.c. of a two per cent. solution of casein in formic acid we add two to three c.c. of this cobalt chloride solution, we obtain a blue-purple mixture. On adding water to this mixture it changes in color from blue-purple, through red-purple to clear pink. Not until a pure pink color is obtained does a precipitate result. If, instead of adding water, we add a considerable volume of alcohol (ten volumes) the mixture rather abruptly changes to a clear pale blue, and then, but not before, we obtain a coagulum of cobalt caseinate.

Electronegative casein (*i. e.*, casein dissolved in alkalies) is not precipitated by the salts of the alkalies, although it is readily precipitated by salts of the alkaline earths. Electropositive casein (*i. e.*, casein dissolved in acids) is, however, very readily precipitated by salts, and these precipitates are not soluble upon dilution. Thus if two c.c. of tenth normal hydrochloric acid be added to five cubic centimeters of a one per cent. solution of casein in 0.008 N. potassium hydroxide, a clear, acid solution of casein results. The casein is precipitated from this solution by the addition of four drops of a saturated solution of sodium chloride, or by one drop of a saturated solution of ammonium sulphate. This latter precipitate does not dissolve on diluting the mixture to one-sixteenth.

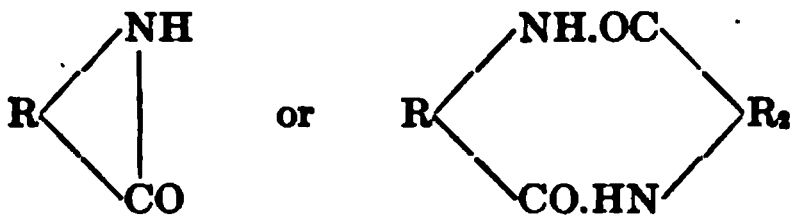
Casein Formate affords no exception to the rule that salts of casein with acids are precipitable by relatively small concentrations of neutral salts, but the *precipitation will only occur in the presence of a sufficiency of water.* If to five cubic centimeters of a two per cent. solution of casein in formic acid we add a saturated solution of ammonium sulphate, three cubic centimeters of this solution just suffice to produce a coagulum, this becomes more abundant on adding water, and redissolves on adding formic acid. If, however, instead of adding three we add two cubic centimeters of the saturated ammonium sulphate solution, a clear solution is obtained. *On adding water to this a precipitate results which redissolves on heating and reappears on cooling.*

Analogous results may be obtained with **Ovomucoid.**

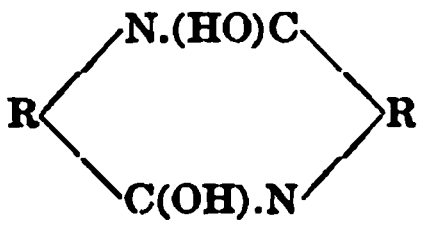
It is clear, therefore, that protein may be thrown out of solution by electrolytes in two grades of hydration, the one of high, the other of very low hydration. The former process is what we have termed **Precipitation,** the latter we have defined as **Coagulation.** At grades of

hydration intermediate between these extremes the protein may be soluble. Dehydration, partial or complete, leading to resolution or to coagulation may be induced by heat, by non-electrolytes possessing an affinity for water, or by concentrated electrolytes.

The importance of a high degree of dehydration in the production of **Coagula**, irresistibly suggests that this phenomenon is dependent upon the formation of anhydrides, analogous to the **Diketopiperazines** which may be formed from the amino-acids and polypeptides by dehydrating-agents, and of the general formula:



Such bodies may exist either in the keto-form, illustrated by the above formulæ, or in the enol-form, such as:



Coagulation by mineral salts appears invariably to be accompanied or preceded by chemical interaction of the coagulating-salt and the protein salt of an acid or base which preëxisted in solution before the coagulant was added. The coagulated protein in these instances, therefore, does not represent the unaltered protein salt as it existed in solution before the coagulant was added. When **Alcohol** is used as the coagulant however, it is found, at least in the case of the **Caseinates of the Alkaline Earths**, that the protein salt as such is coagulated, carrying down with it the amount of mineral base with which it was combined before the coagulant was employed, so that after washing out the alcohol with ether, and absorbing the ether by desiccation over sulphuric acid, calcium caseinate is obtained in the form of a dry powder which is soluble in water, whereas free casein is insoluble in water. If coagulation by alcohol is attributable to dehydration of the protein, the elements of water must be contributed chiefly by the interaction of free amino- and carboxyl-groups with the formation of ring-anhydrides, and that this should be possible without disintegration of the compounds with bases affords another indication that free carboxyl-groups are not responsible for the union of proteins with bases. The same considerations probably will be found to apply to the coagulation by alcohol of the compounds of proteins with acids, but as yet these compounds have not been so thoroughly investigated from this standpoint as the compounds of proteins with bases.

COMPOUNDS OF PROTEINS WITH OTHER PROTEINS.

When the **Protamines**, which, it will be recollected, are strongly basic proteins, are added to weakly alkaline solutions of other proteins, precipitates are formed which consist of compounds of the protamine and other protein employed. These compounds, once formed, are tolerably stable, and when precautions are taken to prevent admixture with excess of protamine they are found to be of very constant composition. These compounds were investigated by Hunter who found that while crystallized egg-albumin, casein, hemi-elastin, gelatin, edestin, heteroalbumose, protalbumose, "alkali albuminate" and histone sulphate yield a precipitate in alkaline solutions upon the addition of the protamine **Clupeine**. Elastin-peptone, deuteroalbumose histopeptone and several peptides fail to yield a precipitate. On digestion of these precipitates with **Pepsin** the protamine is set free, since the protamines are indigestible by pepsin, and the remainder of the compound is converted into proteoses and peptones.

The compound of **Clupeine** with casein contains six per cent. of the protamine while the compound with hemoglobin contains five per cent of protamine. The compound of **Salmine** with edestin contains about ten per cent. of the protamine.

When **Globin** and **Casein** are mixed in faintly acid solution a precipitate of globin caseinate is formed which is soluble in excess of acid or in dilute alkalies. The precipitate produced by admixture of an excess of globin with sodium caseinate in solution contains about 34.5 per cent. of casein. A compound of globin with deuteroalbumose has also been prepared by C. L. A. Schmidt.

Thymus-histone combines with **Hemoglobin**, according to af Ugglas, in the proportion of one part of thymus-histone to two of hemoglobin, and with casein to form a compound containing about thirty per cent. of histone.

A particularly interesting compound protein is the **Hemoglobin Caseinate** which has been prepared by af Ugglas. To a solution of casein in alkali an excess of hydrochloric acid is added until the precipitate of free casein which is at first formed is redissolved. The casein hydrochloride is precipitated from this solution by the addition of sodium chloride, and the precipitate redissolved and reprecipitated until the washings from the precipitate are perfectly neutral. A solution of this substance added to an excess of a solution of hemoglobin produces a precipitate containing 33 per cent. of casein and about 66 per cent. of hemoglobin. The commonly accepted molecular weight of hemoglobin, originally deduced from its content of iron, and now confirmed by a variety of measurements, is about 16,700. The minimal molecular weight of casein, calculated from the minimal quantity of an alkali which will just carry it into solution (see p. 154), is 8800. It seems evident, therefore, that casein and hemoglobin combine with one another in molecular proportions. If the same is

true of the compounds of the various **Protamines** with such proteins as casein and hemoglobin, the low proportion of protamine which is present in these compounds would indicate that the protamines possess molecular weights in the neighborhood of 800; much lower, that is, than the weights of the majority of protein molecules. This corresponds with their less colloidal character, the compound **Salmine** with sulphuric acid, for example, being freely diffusible through parchment-paper, and with the relatively few amino-acids they yield on hydrolysis, reminding one of the peptones rather than of the more bulky and complex native proteins.

From a variety of observations it appears extremely probable that many of the protein constituents which may be isolated from the various tissues and tissue-fluids do not preëxist there, but represent fractions split off by chemical procedures from complex compounds of proteins with proteins which are present in the tissue or tissue-fluid. Thus W. B. Hardy has pointed out that in untreated **Blood-serum** no proteins exist which wander in an electrical field, but as soon as the serum is acidified with acetic acid, a cloud appears, which is due to partial precipitation of "**Insoluble**" **Serum-globulin** (the globulin-fraction of serum which is insoluble in distilled water). On passing a current through this mixture the cloud moves over to the anode. If the serum be dialyzed until all of the serum-globulin has been precipitated the remaining protein is now found to be completely ionic and is precipitated, on passing a current, at the anode. Dialysis, therefore, or acidification of blood-serum evidently accomplishes the detachment of a fraction ("insoluble" serum-globulin) from the protein-complex which preëxists in untreated serum. This fraction is electrically dissociated and so is the remainder from which it is split off, but the original protein-complex is not dissociated at all.

Moreover, as Hardy has also pointed out, the globulin which we separate by dialysis or by acidification and dilution from blood serum possesses very different physical characteristics from any which are displayed by the proteins in unmodified blood-serum. In Hardy's words: "The globulin-fraction has an abiding characteristic. In all its solutions its molecular state is so gross as to cause the molecules to be arrested by a porous pot. They will not pass such a filter even under pressure. In this it is sharply distinct from the parent serum-protein, which is readily filtrable. If globulin be present as such in serum it is not only non-ionic, but the agent which dissolves it must be something more than alkali and salt, since either alone or together they will not produce so high a grade of solution."

"The difference in the molecular grade of globulin when once separated, and the electrical homogeneity of serum-protein and of the fraction (still capable of further subdividion by salting-out) which remains after the alkaline globulin fraction which most readily appears, has been removed, suggests that serum-protein is a complex unit. If such a unit exists it is not saturated with globulin. Fresh ox-serum has

an extraordinary power of dissolving globulin, it will take up almost its own volume of the thick cake at the bottom of a centrifuge tube; and in ox-serum so saturated there is not a trace of alkali-globulin nor of any ionic protein."

The phenomena observed by Hardy appear to admit of interpretation by the view that the protein-complex in serum is formed by the union of a number of alkali-protein compounds, the union taking place in a manner analogous to that which occurs between proteins and inorganic salts, protein-acid or protein-base compounds in this instance taking the place of the inorganic salts. The soluble compounds which are thus formed are non-ionic, as evidenced by lack of motion in an electrical field.

It has been suggested that the tissues and tissue-fluids of the various species of the animal kingdom may owe their specific and individual character to a characteristic structure of the protein-complexes in their tissues or tissue-fluids. We shall have occasion to revert to this possibility in a subsequent chapter (Chapter XIV).

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CHAPTER IX.

THE NUCLEIC ACIDS AND THE NITROGENOUS BASES.

THE DECOMPOSITION-PRODUCTS OF THE NUCLEIC ACIDS.

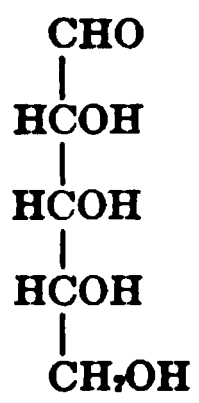
The nucleic acids form the prosthetic group in an important series of conjugated proteins, the **Nucleoproteins.** These substances usually, but not invariably, occur in nuclear tissues and may be precipitated from tissue-extracts by acidification with acetic acid, in excess of which they do not dissolve. The nucleoproteins dissolve, however, in dilute mineral acids and in dilute alkalies; they are not soluble in distilled water. Certain nucleoproteins designated the **β-nucleoproteins,** however, are soluble in boiling water and are extracted from tissues in this manner, leaving the other tissue-proteins in the form of coagula in the insoluble residue; the β-nucleoproteins are also precipitable by acetic acid.

When the alkali-compound of a nucleoprotein, dissolved in water, is heated, a portion of the protein is split off in a coagulated form, while the residue of the molecule, which still contains protein but is much richer in phosphorus than the original nucleoprotein, remains in solution. A similar cleavage is brought about by the **Pepsin** in gastric juice, which digests the protein fraction which is split off from the nucleoprotein, but leaves a residue undigested which still contains protein united to nucleic acid. This residue is designated **Nuclein.**

By means of more intense hydrolysis with alkali the nucleins are split up into products of protein hydrolysis and the alkali salts of the nucleic acids. These salts may be precipitated from concentrated solutions by the addition of alcohol.

Upon hydrolysis with acids all of the nucleic acids yield three widely differing groups of products. In the first place phosphoric acid is an essential constituent of the molecule, secondly a carbohydrate radical, which may be either a pentose or a hexose, and thirdly a nitrogenous base belonging to the group of **Purine Bases** or to the closely allied group of **Pyrimidine Bases.**

The **carbohydrate** radical differs essentially in nucleic acids of different origin. In all of the plant-nucleic acids which have been investigated, the carbohydrate radical has been found to be a pentose d-ribose:



which, until its discovery among the decomposition-products of nucleic acids, was unknown in nature. It is now not only recognized as the carbohydrate radical of plant nucleic acid, but also regarded as the only pentose which normally occurs in *animal* tissues. In two nucleic acids found in animal tissues, but possibly traceable to a vegetable origin, namely **Inosinic Acid and Guanylic Acid**, d-ribose also constitutes the carbohydrate radical, but the nucleic acid which is most characteristic of animal tissues, **Thymus Nucleic Acid**, so called because of the circumstance that it was first prepared in a pure condition from the tissues of the thymus, yields **Levulinic Acid** on hydrolysis by acids. Now levulinic acid, or β-acetyl propionic acid:

$$CH_3CO.CH_2.CH_2.COOH$$

is formed when **Hexoses** are boiled with mineral acids, **Formic Acid** being produced at the same time:

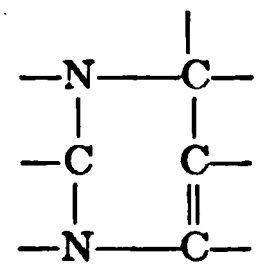
$$C_6H_{12}O_6 = C_5H_8O_3 + HCOOH + H_2O$$

In the hydrolysis of thymus nucleic acid by mineral acids, formic acid is produced as well as levulinic acid. It is evident, therefore, that both of these products are derived from a **Hexose** radical in the nucleic acid and confirmation of this inference is supplied by the fact that on oxidation of thymus nucleic acid with nitric acid, **Saccharic Acid** is included among the products, and saccharic acid must have a hexose precursor:

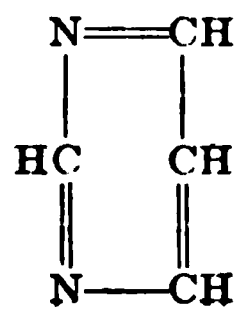
$$2C_6H_{12}O_6 + 3O_2 = 2C_6H_{10}O_8 + 2H_2O$$

Among the **Nitrogenous Bases** which result from the acid hydrolysis of nucleic acids, **Guanine** and **Adenine**, which are **Purine Bases**, are found in both animal and plant nucleic acids, but among the **Pyrimidine Bases** which are yielded by the two classes of nucleic acid, there is a difference, for while both types of nucleic acid yield **Cytosine**, the animal nucleic acid ("thymus nucleic acid") yields **Thymine**, and vegetable nucleic acid yields **Uracil**.

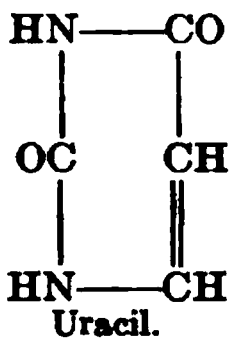
The pyrimidine bases are heterocyclic compounds which are distinguished by the possession of the following nucleus:



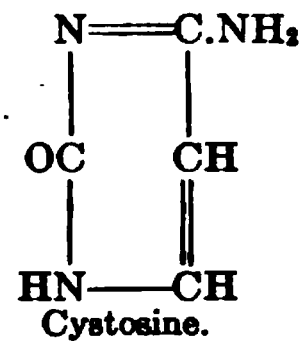
Pyrimidine itself has the formula:



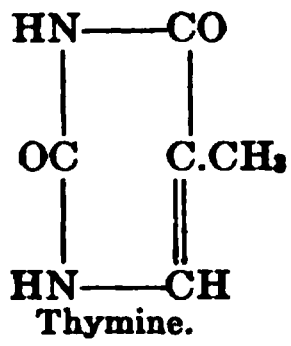
It does not occur among the decomposition-products of the nucleic acids. Its derivatives **Uracil, Cytosine** and **Thymine** have the following formulæ:



Uracil.



Cytosine.

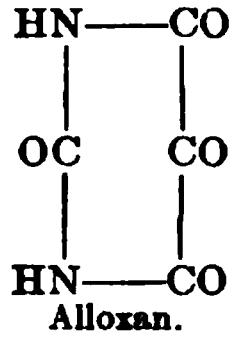


Thymine.

Uracil is therefore dioxypyrimidine, cytosine is amino-oxypyrimidine and thymine is methyluracil. Cytosine is transformed into uracil by the action of nitrous acid.

Each of these bases has been prepared synthetically; they are known to occur in Nature, however, only as decomposition-products derived from nucleic acids by hydrolysis. They are sparingly soluble in cold water, more soluble in hot water. Cytosine dissolves in alcohol, uracil with difficulty, and thymine not at all. Cytosine and thymine are precipitated by phosphotungstic acid. Uracil is not. On heating, thymine sublimes without decomposition, uracil partly decomposes and partly sublimes, while cytosine undergoes decomposition.

Cytosine and uracil give the **Weidel Reaction** as follows: To a small quantity of solution chlorine water is added, and the mixture boiled. The solution is evaporated to dryness and then exposed while warm to the vapors of ammonia. A purple-red color develops. This reaction is frequently referred to as the **Murexide Reaction** because it is due to the formation of **Ammonium Purpurate** which is believed to be identical with the scarlet dye found in the mollusc *murex* which furnished the "purple" of the ancient Romans. An intermediate stage in the reaction is the formation of **Alloxan.**:

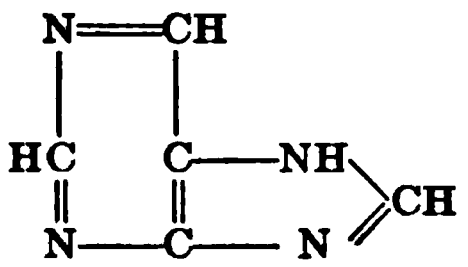


Alloxan.

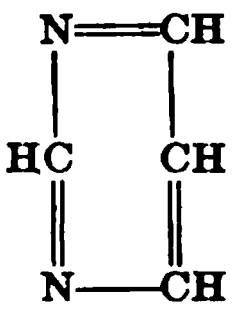
and the test is only given by such substances as can be made to yield alloxan by oxidation. The reaction is therefore not infrequently alluded to as the **Alloxan Reaction.** Nitric acid may be used in the place of chlorine as the oxidizing agent.

Cytosine and uracil also give **Wheeler and Johnson's Reaction**: To the solution of the substance bromine water is added drop by drop until a permanent cloudiness appears. Baryta water is then added,

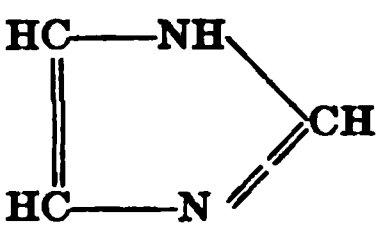
when a purple or violet precipitate appears. The **Purine Bases** are formed by the union of a pyrimidine nucleus with an **Iminazolyl** radical:



Purine.

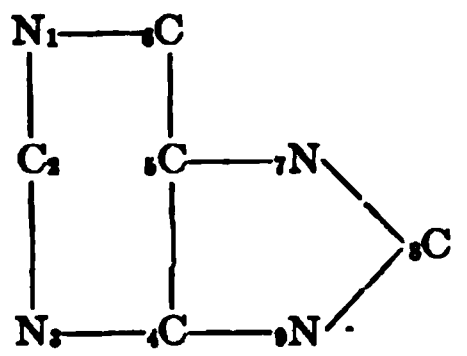


Pyrimidine.



Iminazole.

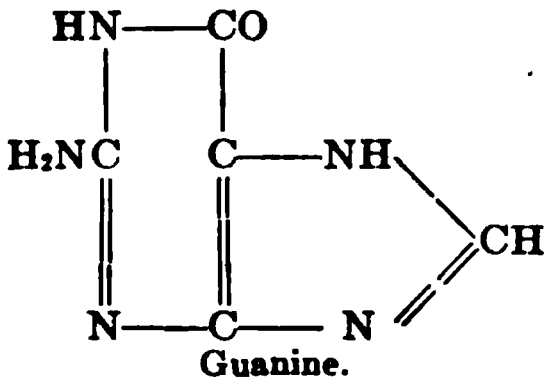
The purine bases which are obtained from the nucleic acids represent only two members of a large group of substances which includes **Uric Acid, Caffeine** and **Theobromine.** For convenience of reference the carbon and nitrogen atoms in the central complex are often numbered as follows:



The purine substances which are most important from a physiological point of view are **Uric Acid, Xanthine, Guanine, Hypoxanthine** and **Adenine,** while **Caffeine, Theobromine** and **Theophylline** are also of importance from a medical and dietetic point of view. Their structure is as follows:

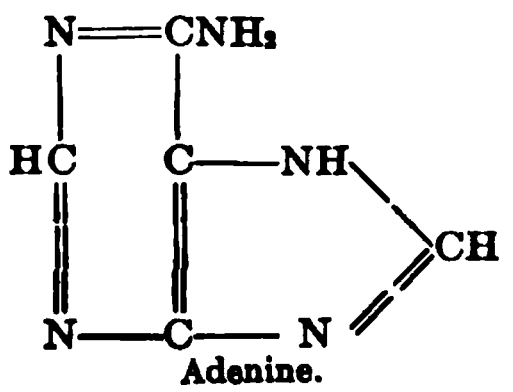
Uric acid	**is**	**2, 6, 8, trioxypurine**
Xanthine	**"**	**2, 6, dioxypurine**
Guanine	**"**	**2, amino, 6, oxypurine**
Hypoxanthine	**"**	**6, oxypurine**
Adenine	**"**	**6, aminopurine**
Caffeine	**"**	**1, 3, 7, trimethyl, 2, 6, dioxypurine**
Theobromine	**"**	**3, 7, dimethyl, 2, 6, dioxypurine**
Theophylline	**"**	**1, 3, dimethyl, 2, 6, dioxypurine**

Thus the formula for **Guanine** may be graphically represented:



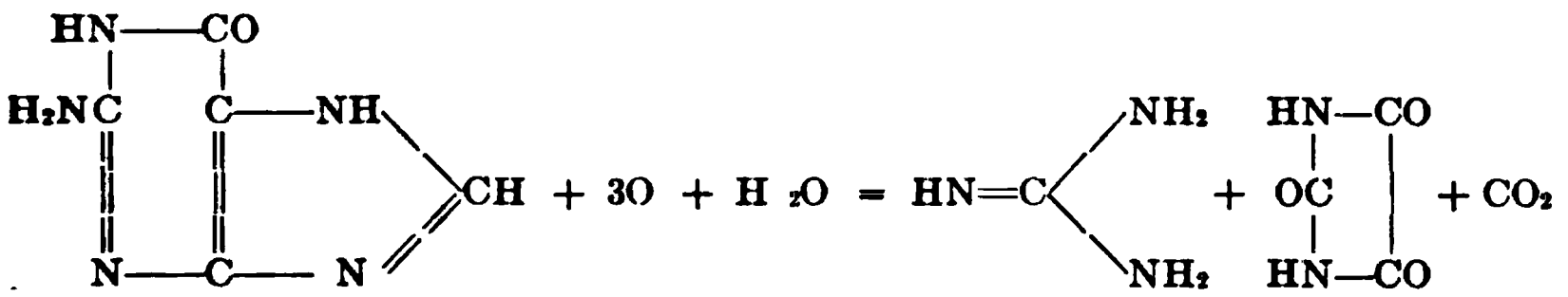
Guanine.

while that of **Adenine** is as follows:



Adenine.

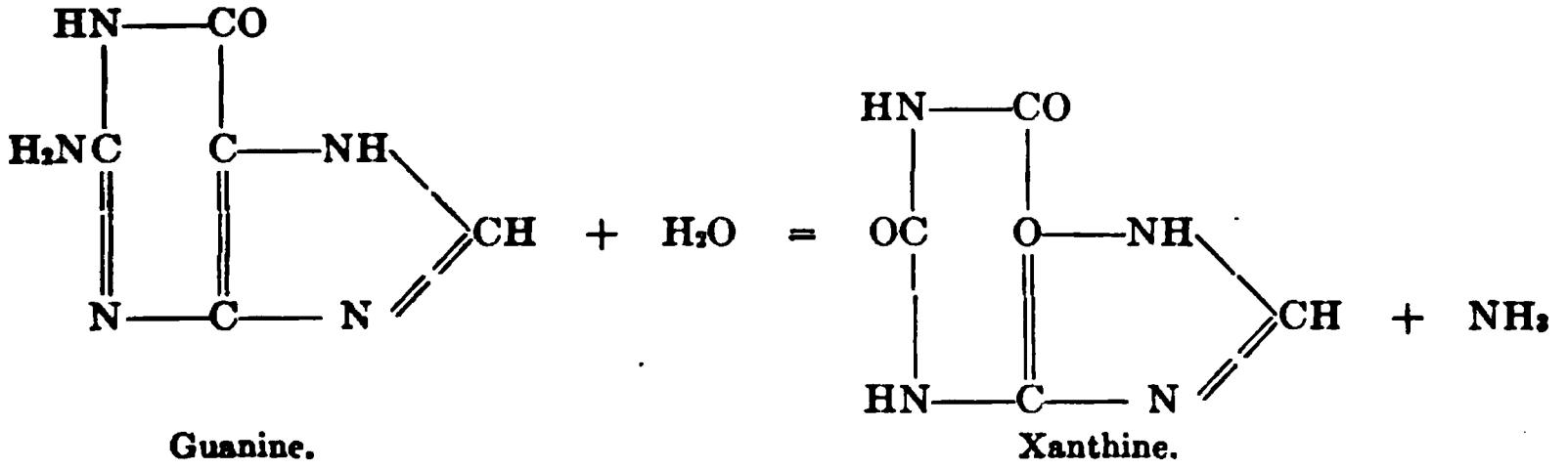
The purine bases are all precipitable from acid solutions by phosphotungstic acid or from ammoniacal solutions by silver nitrate. Guanine is insoluble in water, alcohol or ether, but readily dissolves in dilute acids or alkalies (with the exception of ammonia). It does not give Weidel's reaction, but with nitric acid it yields, on evaporation, a yellow residue which turns bluish-violet on heating with sodium hydroxide. With chlorine water guanine decomposes, yielding **Guanidine, Parabanic Acid** and carbon dioxide:



Guanine. **Guanidine.** **Parabanic acid.**

Free guanine is found in the scales and swimming-bladder of fishes. It also occurs occasionally in the form of concretions in the retinal epithelium of fishes and in the joints of pigs suffering from "guanine gout." It forms an important constituent in the excrement of spiders.

Guanine is hydrolyzed by an enzyme, **Guanase** which is found in a variety of tissues, particularly those of the pancreas and thymus (but not the spleen). The products are **Xanthine** and ammonia:



Guanine. **Xanthine.**

Adenine undergoes an analogous change with the production of **Hypoxanthine,** but the enzyme which brings this about (**Adenase**) appears to be a different one from that which accomplishes the deamination of guanine, since it occurs in the spleen, from which guanase is absent. Hence when the tissues of the thymus or pancreas are allowed to undergo **Autolysis**; that is to say, spontaneous hydrolysis by their own enzymes, the purines which are isolated from the mixture are the oxypurines, xanthine and hypoxanthine, instead of the aminopurines, guanine and adenine which result from hydrolysis by acids.

Adenine is sparingly soluble in cold, but readily soluble in hot water; it is readily soluble in acids and alkalies. Adenine does not give Weidel's reaction. With nitric acid, on evaporation, it gives a nearly

colorless residue which does not turn red or violet on heating with alkali. With hydrochloric acid and zinc and subsequent addition of alkali an adenine solution yields a ruby-red color which changes to a brownish tinge. Adenine has been obtained from certain pathological urines (leukemia) and it occurs in considerable amounts in tea-leaves.

THE STRUCTURE OF THE NUCLEIC ACIDS.

The nucleic acid of yeast appears to be identical with the nucleic acid of the wheat-kernel, **Tritico-nucleic Acid.** It yields, on complete hydrolysis, two purine bases and two pyrimidine bases, namely, guanine, adenine, cytosine and uracil.

When yeast nucleic acid is heated in neutral solutions under pressure to 175° C. it splits off phosphoric acid and yields four different **Nucleosides** each consisting of a molecule of purine or pyrimidine base united to a molecule of α-ribose. These nucleosides are the following:

Guanosine	$C_5H_9O_4.C_5H_4N_5O$
Adenosine	$C_5H_9O_4.C_5H_4N_5$
Cytidine	$C_5H_9O_4.C_4H_4N_2O$
Uridine	$C_5H_9O_4.C_4H_3N_2O_2$

It follows that in the undecomposed molecule of nucleic acid the purine and pyrimidine bases must be attached directly to the α-ribose molecules. The nucleosides do not reduce **Fehling's Solution** and hence the carbohydrate radical must be united to the basic radical in such a way as to involve destruction of the actual or potential aldehyde structure of the sugar, in other words these compounds are analogous to the **Glucosides.**

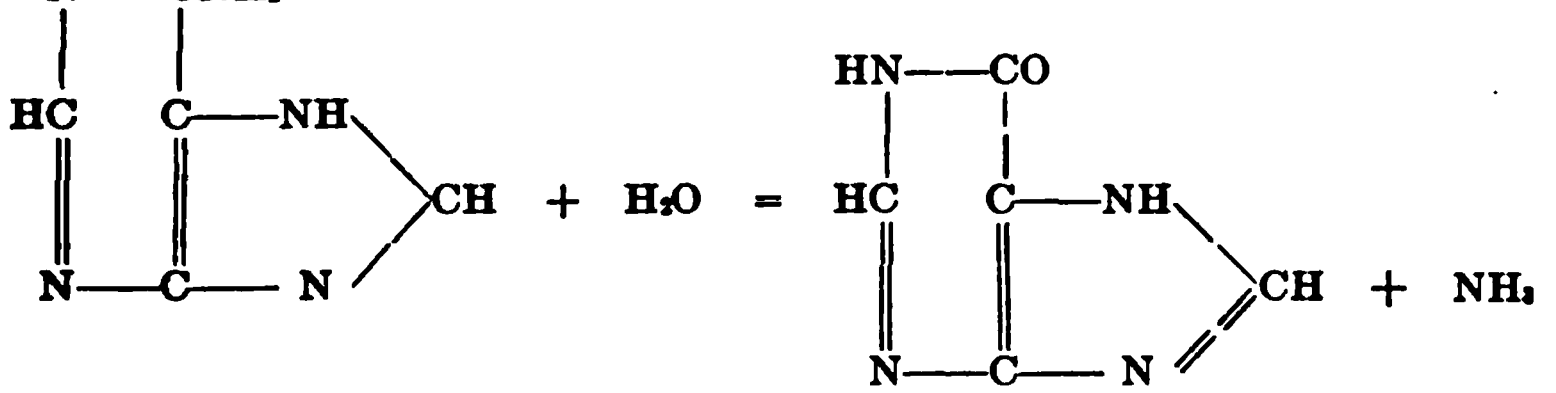
If, instead of hydrolyzing nucleic acid with the aid of heat or inorganic catalyzers, we employ extracts of various organs, such as the kidney, heart-muscle, liver, pancreas, or intestinal mucosa, or if we employ blood-serum or hemolyzed blood, all of which contain the enzyme **Nuclease**, the nucleic acid is split into four different **Mononucleotides** each of which, on intense hydrolysis, yields phosphoric acid, a carbohydrate which in the case of yeast nucleic acid is α-ribose, and one of the four different purine and pyrimidine bases which the original molecule contained. The molecule of nucleic acid is, therefore, a **Tetranucleotid,** built up out of the union of four mononucleotid radicals.

Two mononucleotids are known to occur in animal tissues, they are **Guanylic Acid**, obtained by the partial hydrolysis of β-nucleoproteins, those nucleoproteins which may be extracted from a variety of tissues by **Boiling Water,** and **Inosinic Acid** which exists as such in most extracts.

When guanylic acid is completely hydrolysed by mineral acids it yields phosphoric acid, α-ribose and guanine. It yields no other purine base and no pyrimidine bases. By means of hydrolysis in

neutral water under pressure, phosphoric acid may be split off from this substance and **Guanosine** or the nucleoside of guanine is produced. The **β-Nucleoproteins** are, therefore, compounds of protein with a mononucleotid, while the normal or **α-Nucleoproteins** are compounds of protein with a tetranucleotid.

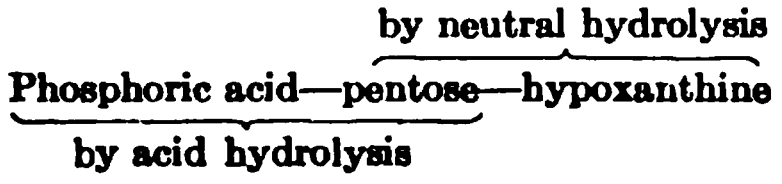
Inosinic Acid is prepared from meat-extracts by converting it into the barium salt which is very sparingly soluble in water. On hydrolysis with acids it yields phosphoric acid, α-ribose and **Hypoxanthine** in molecularly equivalent proportions. It will be recollected that hypoxanthine may be derived from adenine by simple deaminization:



Adenine. **Hypoxanthine.**

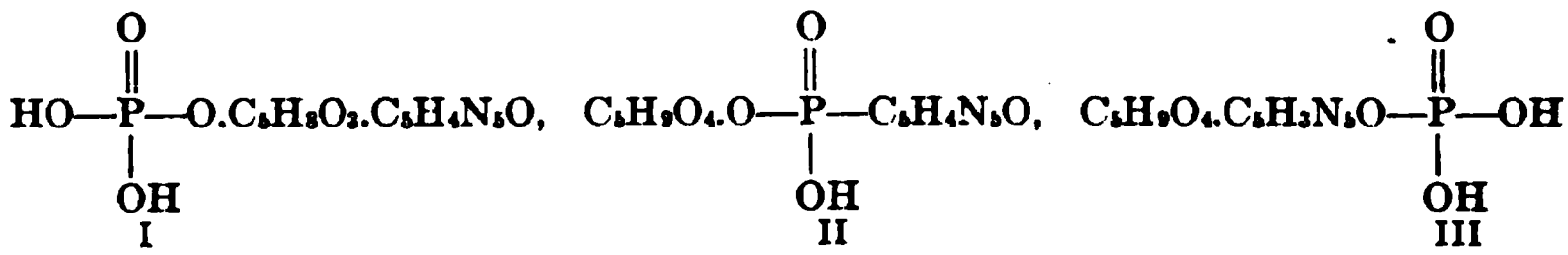
so that inosinic acid is a derivative of a simple mononucleotid containing adenine. The fact that the mononucleotids in animal tissues yield α-ribose on hydrolysis while the tetranucleotid, **Thymus Nucleic Acid**, which is characteristic of animal tissues yields levulinic acid which must be derived from a hexose radical, leads us to infer that the mononucleotids which are found in animal tissues are derived from a vegetable source and are possibly not synthesised by animal tissues at all, but formed by partial hydrolysis and subsequent modification of plant nucleic acids received in the food.

By very careful hydrolysis with acids, interrupting the process before it is complete, it is possible to split off hypoxanthine from inosinic acid, leaving a compound of phosphoric acid and pentose. On the other hand, by neutral hydrolysis under pressure, phosphoric acid is split off leaving the pentose combined with hypoxanthine. It is evident, therefore, that in this mononucleotid the carbohydrate radical occupies a middle position, linking together the phosphoric acid on the one hand and the purine base on the other. This will be clear from the following schema:



we shall see that the arrangement of the radical in other mononucleotids is probably of the same type.

There are three conceivable arrangements of the three constituent radicals of **Guanylic Acid.** They are:



Of these three arrangements II cannot be the one which actually occurs in guanylic acid, because on neutral hydrolysis under pressure it yields **Guanosine** $C_5H_9O_4.C_5H_4N_5O$, which would be impossible if, in the original molecule, the carbohydrate and basic radicals were separated by the interposition of phosphoric acid. Either I or III must be the correct formula.

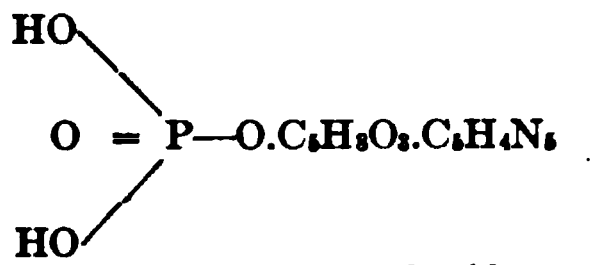
Now on comparing the rates at which phosphoric acid and guanine are liberated from guanylic acid by acid hydrolysis, it is found that guanine is liberated much more rapidly than phosphoric acid. This implies, of course, that during the progress of hydrolysis, while guanine is being split off, phosphoric acid is being held in combination with some other substance from which compound it is detached with relative difficulty. The only substance, guanine being excluded, with which the phosphoric acid can be combined is α-ribose. It follows, therefore, that phosphoric acid is attached to the molecule through the pentose radical, and formula I must represent the actual arrangement of the groups in guanylic acid. The same reasoning applies to the adenine-uracil dinucleotid which may be split off from yeast nucleic acid by partial enzymatic hydrolysis. We infer, therefore, from these facts and from the general similarity of the various mononucleotids to one another that the arrangement of radicals in all of them is:

Phosphoric acid — carbohydrate — purine or pyrimidine.

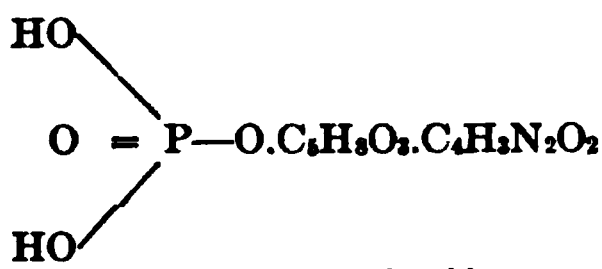
It remains to be considered how these mononucleotid radicals are united together to form the tetranucleotids characteristic, respectively, of vegetable and animal tissues.

Three alternative possibilities exist, namely, (*a*) that the mononucleotids are united to one another through their phosphoric acid groups, so that the tetranucleotid would be a substituted polyphosphoric acid. This was the view originally propounded by Kossel and has claimed very general acceptance until quite recently; (*b*) that the mononucleotids are united to one another through their carbohydrate radicals and (*c*) that they are united to one another through their purine or pyrimidine radicals. Between the two latter alternatives it has not as yet proved possible to decide with certainty, but the first alternative, that the mononucleotids are united to one another through their phosphoric acid radicals, may be dismissed for the following reasons:

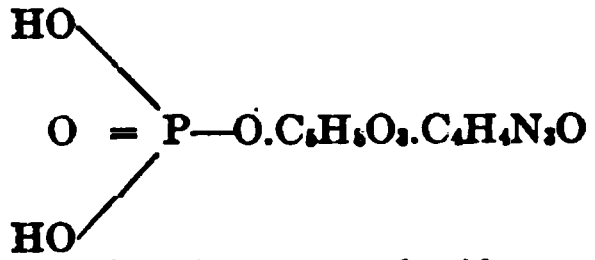
Yeast nucleic acid is known to consist of the following four mononucleotids.



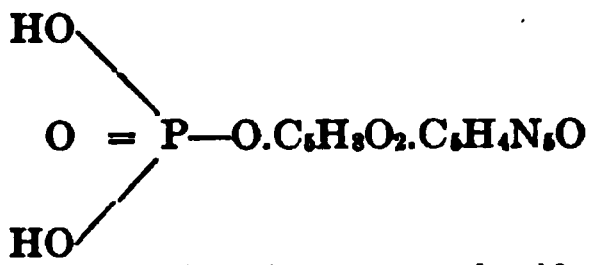
Adenine mononucleotid



Uracil mononucleotid



Cytosine mononucleotid



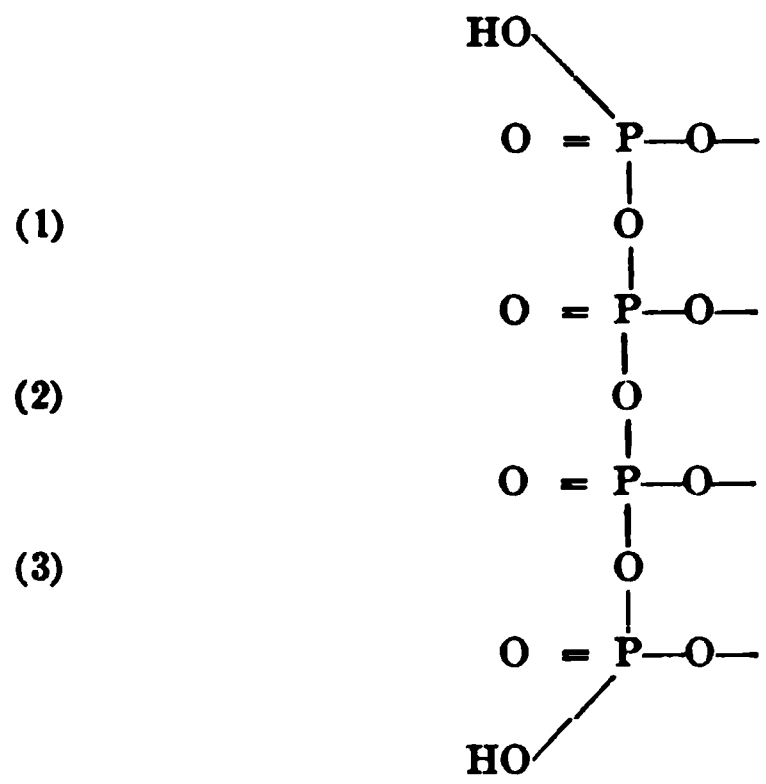
Guanine mononucleotid

We know, also, that these mononucleotids are united to one another in the order indicated, for when yeast nucleic acid is heated with ammonia it yields adenine-uracil dinucleotid, so that the constituent mononucleotids of this substance must be united together in the unaltered nucleic acid molecule. On the other hand, when carefully heated with acids, yeast nucleic acid splits off adenine and guanine mononucleotids leaving uracil-cytosine dinucleotid. It is evident, therefore, that the uracil and cytosine mononucleotid radicals are united to one another in the yeast nucleic acid molecule, and that the adenine and guanine mononucleotids form the extremities of the molecule.

Now the **Adenine Uracil Dinucleotid** might conceivably consist of two mononucleotids united by their phosphoric acid radicals, or they might be united in some other manner. If they were united by their phosphoric acid molecules, at least *one* of the hydroxyl-groups of the phosphoric acid radicles would disappear by neutralization. The total number of available hydroxyl-groups contained in the two phosphoric acid radicals is four, so that the maximum number of molecules of any base that adenin-uracil dinucleotid could combine with would be four. If any hydroxyl-groups were neutralized by union of phosphoric acid radicals with each other or with other parts of the associated mononucleotid the free hydroxyl-groups would be less than four, and the dinucleotid would, in consequence, neutralize less than four molecules of a base. Now adenine-uracil dinucleotid forms a compound with *four* molecules of **Brucine**. It follows, therefore, that

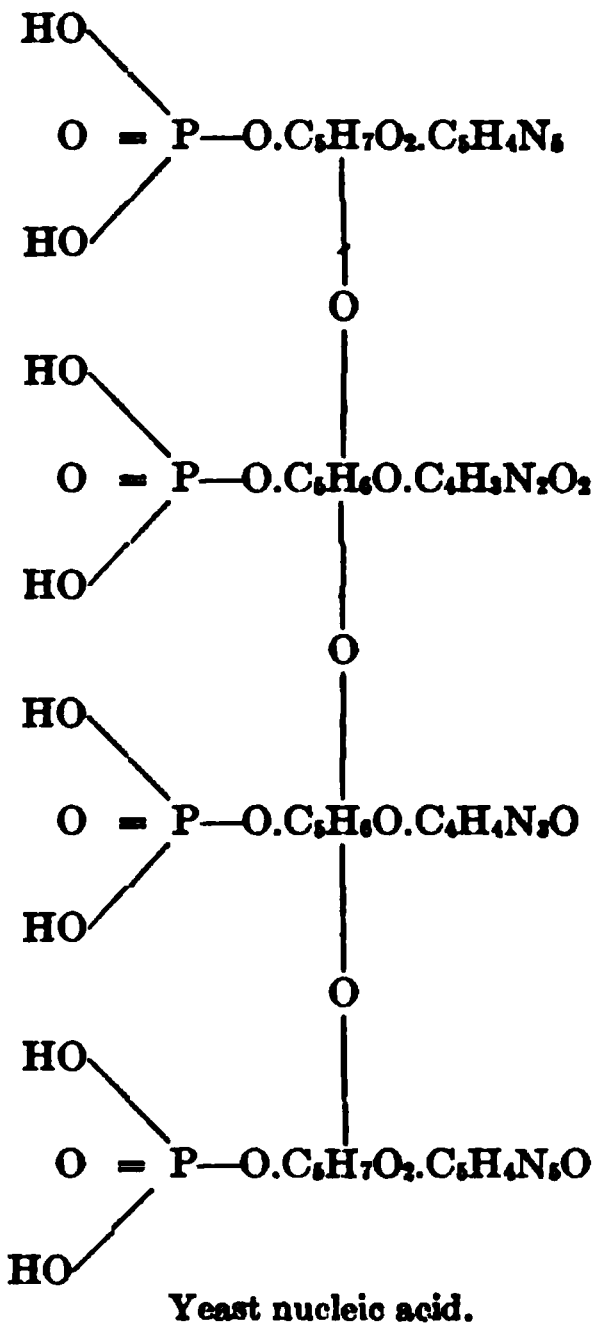
the phosphoric acid radicals of adenine and uracil mononucleotids are not utilized in binding these constituents of the nucleic acid molecule together.

When a **Purine Nucleotid** is heated with dilute sulphuric acid, phosphoric acid is liberated rapidly and completely. On the contrary, when a **Pyrimidine Nucleotid** is similarly treated, phosphoric acid is split off slowly. Yeast nucleic acid yields one-half of its phosphoric acid rapidly, and the remaining half slowly. Now if we compare the relative rates of splitting off phosphoric acid by adenine-uracil dinucleotid and by the whole yeast nucleic acid when treated in this manner, we find the relative rates of yielding phosphoric acid are identical. Hence, so far as phosphoric acid is concerned, the nucleic acid molecule consists of two symmetrical parts. Union of the two dinucleotid fractions to form whole nucleic acid does not in the slightest degree affect the rate of yield of phosphoric acid by the component dinucleotids, and hence, phosphoric acid cannot be concerned in their union, and the phosphoric-acid linkage (2) in the subjoined diagram evidently does not exist in nucleic acid.



The molecule of yeast nucleic acid having thus been shown to consist of two symmetrically constructed halves, so far as phosphoric acid is concerned, it follows that if linkage (3) exists, then linkage (1), which would unite the adenine, and uracil mononucleotids must also exist, but this linkage has been shown not to exist, by the composition of the brucine salt of the adenine-uracil dinucleotid. Hence linkage (3) does not exist either and, in short, no phosphoric-acid linkages exist which bind molecules of mononucleotid together to form the tetranucleotid yeast nucleic acid.

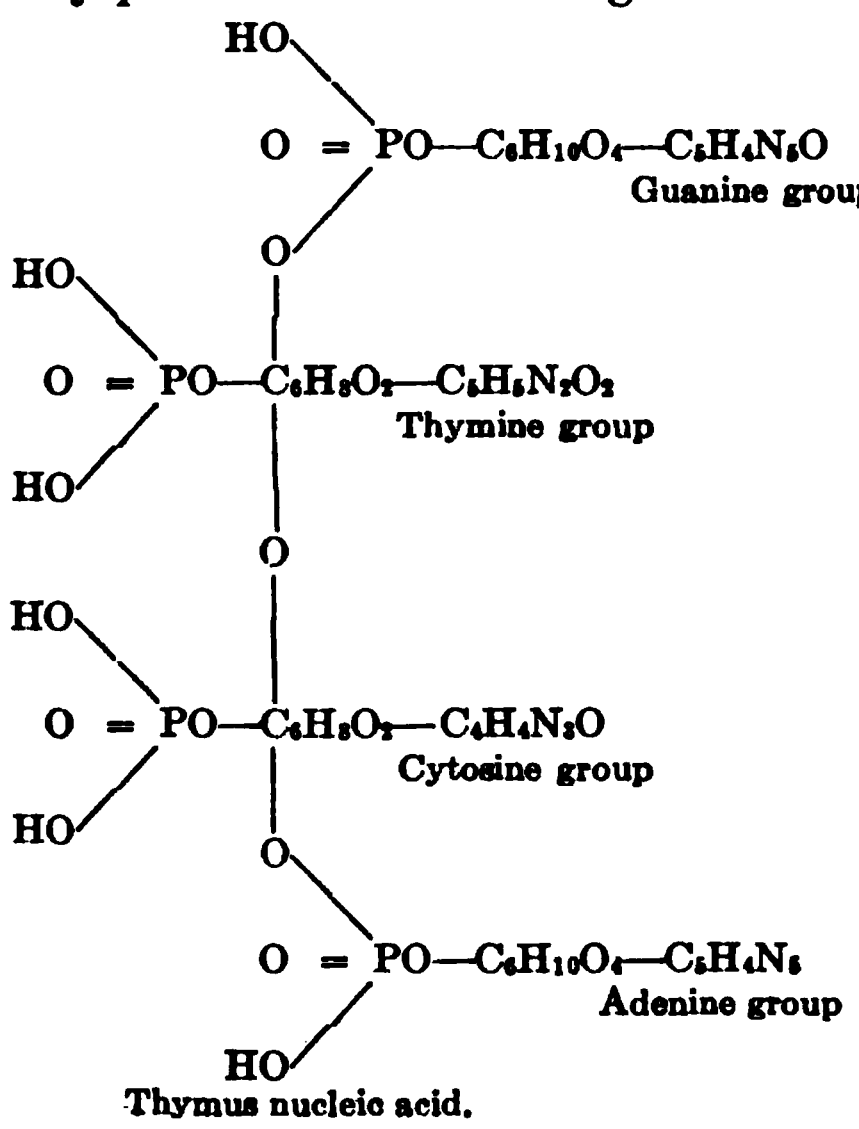
Between the two remaining forms of linkage, by the carbohydrate or by the purine or pyrimidine radical it has not yet been possible to certainly decide. P. A. Levene, however, concludes that in **Cystosine-uracil Dinucleotid** only two possibilities exist, either constituent mononucleotids are connected by ribose to ribose, or else by uracil (not by cystosine) to ribose. W. Jones believes yeast nucleic acid to be constituted as follows:



Yeast nucleic acid.

Tritico-nucleic Acid from the wheat embryo is identical in physical behavior and in the products it yields on hydrolysis, with yeast nucleic acid. They are therefore believed to be identical substances, and it is considered probable that this is the only vegetable tetranucleotid.

Thymus Nucleic Acid, it will be recollected, is yielded by the partial hydrolysis of all nucleoproteins of animal origin. It contains a hexose radical which has not yet been positively identified and it yields thymine instead of uracil. Levene and Jacobs consider that thymus nucleic acid probably possesses the following structure:



Thymus nucleic acid.

AMINES DERIVED FROM AMINO-ACIDS.

The proteolytic enzymes, such as **Trypsin** and **Erepsin**, accomplish the conversion, by hydrolysis, of the proteins into their constituent amino-acids. The next step in the degradation of nitrogenous food-stuffs by animal tissues generally, appears to consist in **Deaminization** with the splitting off of ammonia and the oxidation of the remainder of the original amino-acid molecule to carbon dioxide and water. No intermediate stages in this process have been definitely established, and we have been unable to detect the presence in animal tissues of enzymes capable of producing nitrogenous bases other than ammonia from amino-acids. That such enzymes, perhaps highly localized, do actually exist in animal tissues may be regarded as exceedingly probable, from the variety and physiological importance of the nitrogenous bases which are found to occur in animal tissues and their significant chemical resemblance to certain of the amino-acids which are yielded by the digestion of protein.

Bacteria and other **Fungi**, however, constitute a group of organisms which are able to rapidly produce from amino-acids a series of nitrogenous bases which arise by **Decarboxylization** of the amino-acid molecule in accordance with the general equation:

$$\underset{\text{Amino-acid}}{\begin{matrix}R\\|\\CHNH_2\\|\\COOH\end{matrix}} = \underset{\text{Amine}}{\begin{matrix}R\\|\\CH_2NH_2\end{matrix}} + CO_2$$

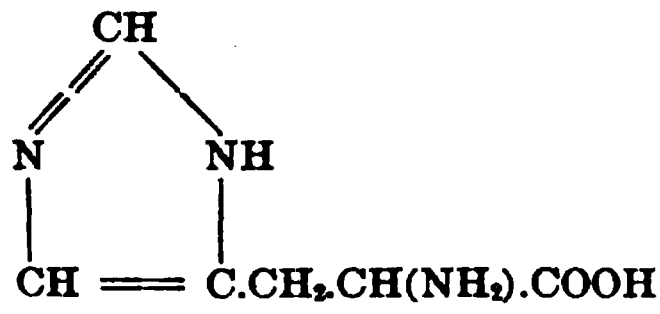
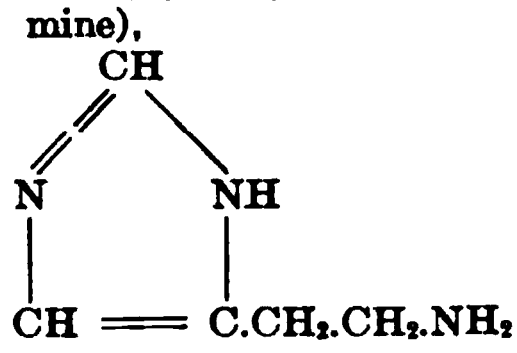
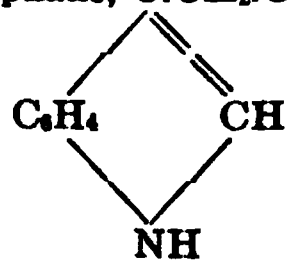
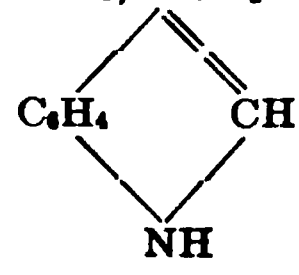
At the same time that this is taking place, **Deaminization** is also proceeding, and is evidenced by the production of ammonia. The conditions determining the relative proportion of these two processes are complex and have not as yet been fully determined, but it has been observed that the presence of carbohydrates in a culture of bacteria or fungi greatly diminishes the production of ammonia, presumably because in the absence of carbohydrates the organisms utilize amino-acids as a source of energy as well as a source of nitrogen, and consuming the carbon and hydrogen components for this purpose, split off ammonia as a by-product. In studying the decarboxylization of individual amino-acids it has been found that the addition of **Peptone** to the bacterial culture increases the yield of amines, probably because the process of deaminization being shared between the amino-acid and the peptone, a greater proportion of the amino-acid remains available for decarboxylization.

Decarboxylization may also, especially under anaërobic conditions, be accompanied by reduction, in which case **Formic Acid** is produced instead of carbon dioxide:

$$\underset{\text{Amino-acid}}{\begin{matrix}R\\|\\CHNH_2\\|\\COOH\end{matrix}} + H_2 = \underset{\text{Amine}}{\begin{matrix}R\\|\\CH_2NH_2\end{matrix}} + HCOOH$$

The greatest importance of this process from a biochemical point of view arises out of the intense physiological activity of many of the products which originate in this manner, the resemblance of some of these products to the active principles of certain of the glands of internal secretion, and from the probability that some of them may reach the circulation, occasionally in injurious quantities, by absorption from the large intestine wherein they are produced by bacterial activity.

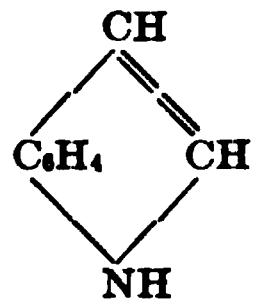
The following amines have been produced from the corresponding amino-acids by the action of putrefactive bacteria. It is possible, however, that the true source of methylamine in the putrefaction of fishes is not glycocoll, but choline (trimethyl oxyethyl ammonium hydroxide) which is the basic constituent of **Lecithin**.

Amino-acid.	**Amine.**
Glycocoll, $CH_2.NH_2.COOH$	Methylamine, CH_3NH_2
Alanine, $CH_3.CH.NH_2.COOH$	Ethylamine, $CH_3CH_2NH_2$
Valine, $(CH_3)_2CH.CH(NH_2).COOH$	Isobutylamine, $(CH_3)_2CH.CH_2NH_2$
Leucine, $(CH_3)_2CH.CH_2.CH(NH_2).COOH$	Isoamylamine, $(CH_3)_2CH.CH_2CH_2.NH_2$
Isoleucine, $CH_3.C_2H_5CH.CH(NH_2).COOH$	Ethylmethyl ethylamine $CH_3.C_2H_5CH.CH_2NH_2$
Phenylalanine, $C_6H_5CH_2.CH(NH_2).COOH$	Phenylethylamine, $C_6H_5.CH_2.CH_2.NH_2$
Tyrosine, $HO.C_6H_4.CH_2.CH(NH_2).COOH$	*p*-Hydroxyphenylethylamine (tyramine), $HO.C_6H_4.CH_2CH_2NH_2$
Arginine, $HN = C(NH_2)(NH.CH_2.CH_2.CH_2.CH(NH_2).COOH)$	Guanido-butylamine (agmatine), $HN = C(NH_2)(NH.CH_2.CH_2CH_2CH_2NH_2)$
Lysine, $H_2N.CH_2.CH_2CH_2.CH_2.CH(NH_2).COOH$	Pentamethylene-diamine (cadaverine), $H_2N.CH_2CH_2.CH_2CH_2.CH_2NH_2$
Ornithine, $H_2N.CH_2CH_2.CH_2.CH(NH_2).COOH$	Tetramethylene-diamine (putrescine), $H_2N.CH_2.CH_2.CH_2.CH_2NH_2$
Histidine, 	Iminazoylyl ethylamine (histamine, ergamine), 
Tryptophane, $C.CH_2.CH(NH_2).COOH$ 	Indolethylamine, $C.CH_2CH_2NH_2$ 

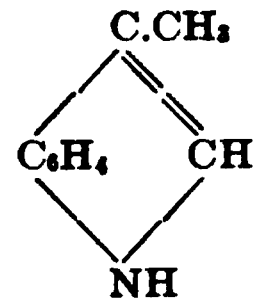
Pyrrolidine, which should be formed by decarboxylization from proline, oxypyrrolidine which should be formed from oxyproline, amino-ethyldisulphide which should be formed from cystine, and β-hydroxyethylamine which should be formed from serin, have not yet been found possible to prepare by bacterial decarboxylization.

While a wide variety of bacilli, especially anaërobes, are able to bring about the decarboxylization of amino-acids, this power would seem to be possessed in an exceptional degree by a specific organism, *Bacillus aminophilus intestinalis* which has been isolated by Bertrand and Berthelot.

The production of these bases, many of which are definitely toxic is not necessarily accompanied by the production of the odor which is commonly considered to be indicative of putrefaction. The *odor* of putrefaction is due to **Indol** and **Skatol** or β-methyl indol:



Indol.



Skatol.

and these substances which are derived from **Tryptophane** are the products of a further stage of putrefactive decomposition, arising by combined decarboxylization and deaminization succeeded by partial (skatol) or complete (indol) oxidation of the aliphatic hydrocarbon chain of the tryptophane molecule.

The bases which are derived in this way from the proteins display the usual characteristic properties of the amines. They are very much more basic than the amino-acids from which they are derived, and yield crystalline salts with mineral acids.

The aliphatic monamines (methylamine, ethylamine, isobutylamine, isoamylamine, dimethylaminobutane) exert a physiological action mimicking the effects of stimulation of the sympathetic nervous system, they are therefore termed by Barger and Dale "**Sympathomimetic**" bases. The lowest amine to produce a distinct rise in blood pressure on intravenous injection is, however, **Isobutylamine**; the activity increases with increasing length of the aliphatic hydrocarbon chain up to **Hexylamine**, and thereafter declines as the number of carbon atoms increases. Very much more effective than mere increase in the length of the chain is, however, the introduction of a ring-structure as in the benzol and heterocyclic derivatives. Thus **Phenylethylamine** is at least five times as active, physiologically, as any aliphatic amine. Two milligrammes of this substance when injected intravenously may increase the blood-pressure of a cat no less than six hundred per cent. (30 mm. to 180 mm.). The most active, however, of the monamines derived from the amino-acid cleavage-products of protein is parahydroxyphenylethylamine (**Tyramine**) which exerts an

effect upon blood-pressure about one-twentieth of that exerted by **Adrenaline**. When injected intravenously it causes a rapid and pronounced rise in blood-pressure which is somewhat more prolonged than the rise which is caused by injections of adrenaline. Unlike adrenaline, however, tyramine does not cause any vasoconstriction when applied locally to mucous surfaces, and large doses fail to produce the glycosuria which results from adrenaline-poisoning. Tyramine, furthermore, has a decided action upon the uterus, causing the non-pregnant uterus to relax while the pregnant uterus is stimulated to contraction. The glands which are innervated by the sympathetic system are stimulated by tyramine.

It has been considered possible that since tyramine may be produced *in vitro* from **Tyrosine** by the action of fecal bacteria, the presence of this substance in the large intestine and its absorption may be responsible for pathological conditions in which high blood-pressure is a leading symptom. As in the case of adrenaline, prolonged administration of tyramine leads to renal and vascular lesions similar to those which so generally accompany persistent arterial hypertension in man.

Indolethylamine is not so potent as tyramine and differs from it in several details of its action, notably in giving rise to muscular tremors or even convulsions, due to a transient stimulation of the central nervous system. Indolethylamine has also a direct stimulatory action on smooth muscle-fibers, which is especially marked in the arterioles of the iris and the uterus.

Among the **Diamines, Putrescine** and **Cadaverine** are of historic interest as they were among the earliest putrefaction-bases to be isolated, definitely characterized and identified. They are, however, comparatively innocuous substances, having very slight physiological activity and in common with other diamines, but in contrast to the monamines, they cause a *fall* in blood-pressure when they are injected intravenously. They occur in the urine in cases of cystinuria, their presence indicating a defective power of the tissues to deaminize amino-acids.

Agmatine has a direct action upon the muscular tissues of the uterus, inducing contractions; it is, however, very much less potent in this respect than **Ergamine** which, with **Ergotoxine** and **Tyramine** is the active principle of the pharmaceutical preparations of ergot.

Ergot is a parasitic fungus, *Claviceps purpurea*, which grows on diseased rye, and has been employed from very ancient times to cause contractions of the uterus. The amines which it contains are undoubtedly produced by this fungus, as they are by other fungi and bacteria, by decarboxylization of the corresponding amino-acids. Ergamine stimulates unstriated muscle-cells directly, inducing especially powerful contractions of the uterus, but also stimulating smooth-muscle fibers in other organs, for example the stomach and intestine and the constrictor muscles of the pupil of the eye. When dissolved in physio-

logical saline solution and perfused through excised bloodvessels the muscle-fibers of the vessels contract, causing a decrease in their diameter, but when ergamine is injected intravenously, the effect upon the majority of the vessels *in situ* is just the reverse, and the blood-pressure undergoes a profound decrease due to their dilation. The vessels of the lungs, heart and kidneys, however, are constricted. An exceptionally interesting action of ergamine is that of inducing spasmodic contractions of the **Bronchioles** when administered in relatively large doses. Thus 0.5 milligrams of ergamine intravenously injected will kill a guinea-pig in a few minutes, and the cause of death is asphyxiation, which is due to closure of the bronchioles, preventing the passage of air into or out of the lungs. Post-mortem examination shows that the lungs are permanently dilated (**Emphysema**). Now this is the condition which, in a milder degree, is responsible, in human beings, for the respiratory distress in **Asthma.** It may further be brought about by peptone-poisoning or by inducing **Anaphylactic Shock.**

When a non-toxic foreign protein, for example egg-white, is injected hypodermically or into the circulation of an animal, if the first dose is followed within a few days by a second, that in a like period by a third, and so forth, no harmful results ensue, and the animal gradually acquires **Immunity** to the protein. If, however, after the injection of the first dose of protein a considerable period, *e. g.*, three weeks, be allowed to elapse before the second is administered, if the second dose be sufficiently large, a condition of "anaphylactic shock" is induced which is frequently fatal. The cause of death is asphyxiation due to spasmodic contractions of the bronchioles and it is believed that the preliminary "sensitization" of the animal has endowed its tissues with the ability to so rapidly decompose the foreign protein that upon injection of the second dose dangerous quantities of toxic peptones or other products of protein decomposition are rapidly formed. The resemblance between the symptoms of ergamine poisoning, peptone poisoning, asthma and anaphylactic shock is so striking as to suggest a common cause and the view has been advanced that all of these phenomena are attributable to the liberation of β-iminazolyl ethylamine in the blood or tissues, the source of the substance being the **Histidine** radical in proteins or peptones. On the other hand it has not been conclusively shown that peptones themselves or peptide-derivatives of β-iminazolyl ethylamine may not produce like effects. At any rate a part of the symptoms of anaphylactic shock are not attributable to ergamine, because this substance does not render blood incoagulable, while incoagulability of the blood is one of the symptoms of profound anaphylactic shock and of peptone poisoning.

The possibility of the formation of β-iminazolyl ethylamine from proteins in the lower intestine by the action of fecal bacteria may enable us to trace certain forms of asthma to an intestinal source. The majority of cases appear, however, to be undoubtedly anaphy-

lactic, the immediate origin of an attack being frequently traceable to ingestion of some protein to which the individual in question has become sensitized, *e. g.*, the proteins in the sweat of horses, egg-white, the proteins in strawberries or in pollen, or possibly proteins produced locally by bacterial infections. On the other hand asthmatic attacks, originally anaphylactic, may frequently be seen in early cases to pass through transitional stages into habitual reflexes, which are thereafter elicited by any unusual stimulus, *e. g.*, emotional excitement or indigestion. The problem is therefore a many-sided one of which the several factors are frequently difficult or impossible to disentangle.

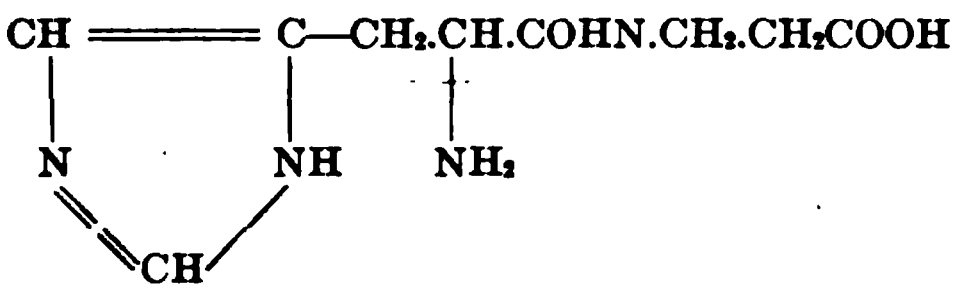
Closely related to the amines which we have been considering are the **ω-Amino-acids** in which the α-amino-group which is so characteristic of the amino-acids derived from proteins is absent, the amino-group being attached to a carbon atom which is remote from the carboxyl-group. This results in greatly increased basicity of the amino-acid so that these compounds resemble the amines in chemical behavior rather than the amino-acids. They may be produced in putrefaction by partial deaminization of a diamino-acid, as in the production of **δ-Amino-valeric Acid** from ornithine:

$$H_2N.CH_2.CH_2.CH_2.CH(NH_2)COOH + 2H = H_2N.CH_2.CH_2.CH_2.CH_2.COOH + NH_3$$

or they may result from partial decarboxylization of a dicarboxylic acid, as in the production of **γ-Aminobutyric Acid**, from glutamic acid:

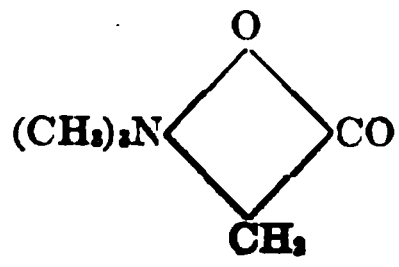
$$HOOC.CH(NH_2).CH_2.CH_2.COOH = H_2N.CH_2.CH_2.CH_2.COOH + CO_2$$

An important representative of this group of substances is **Carnosine** which, next to creatine, is the most abundant nitrogenous base in meat-extracts. It is present in horse-meat to the extent of 1.8 grams per kilo. On hydrolysis it yields **Histidine** and **β-Alanine** in equimolecular proportions. It is believed to be a dipeptide histidyl-β-alanine:

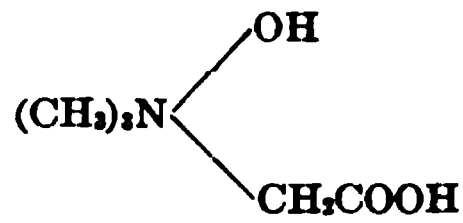


THE BETAINES AND THE VITAMINES.

The **Betaines** are amino-acids in which the nitrogen atom is united to methyl-groups in the place of hydrogen atoms. These substances in the absence of water, form cyclic anhydrides which open up when they are dissolved in water or unite with acids. Thus **Betaine** itself, or **Trimethylglycine** has the formula:

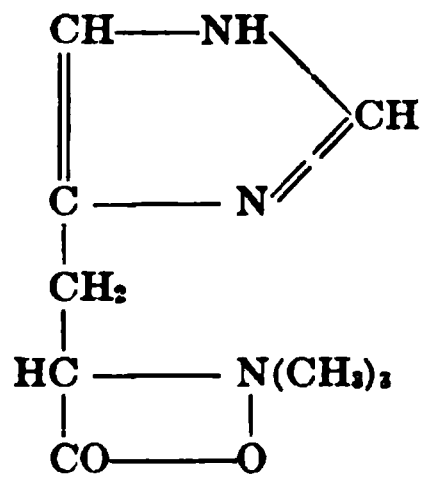


When dried at above 100°; but when it is dissolved in water or combined with acids it is probably represented by the formula:



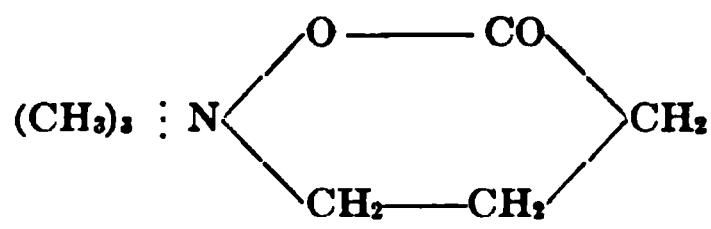
Betaine occurs in the sap of the sugar-beet, *Beta vulgaris*, and is extracted together with the sugar, remaining in the molasses when the sugar is refined. It is non-toxic and is not utilizable by animals as a food, but it is stated that the creatine content of the muscles is perceptibly increased by administration of betaine.

Trimethyl Histidine is found in edible mushrooms. The constitution is:



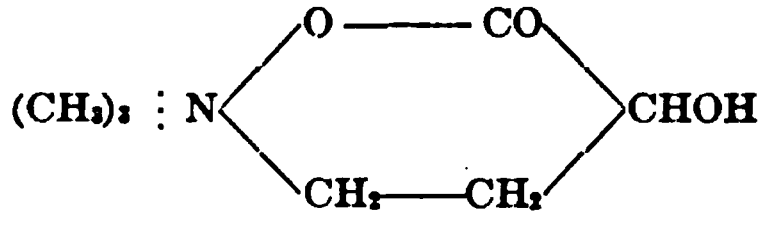
The corresponding betaine of tryptophane is **Hypaphorine.** Up to the present these betaines have only been found in plant-tissues.

In putrefying meat we find a betaine which unlike those described above, has a powerful physiological action. This is γ-n-butyro-betaine, the betaine of γ-amino-butyric acid:



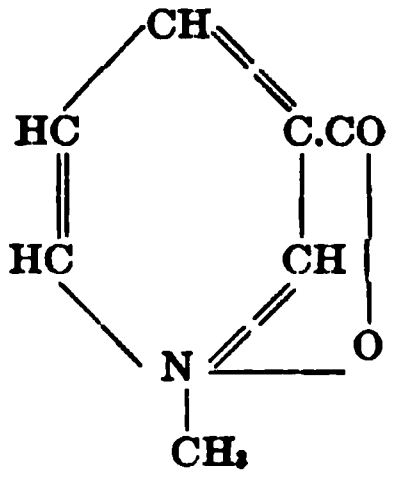
It has an action upon nerve-endings resembling that of curare and when injected produces convulsions, dyspnea and paralysis.

Carnitine is the α-hydroxy derivative of γ-butyro-betaine:



it is found in meat-extracts and is almost devoid of immediate physiological actions.

Trigonelline is the betaine of nicotinic acid and, therefore, unlike the betaines heretofore considered, is not obtainable from any amino-acid cleavage-product of proteins. Its constitution is as follows:



it is devoid of any obvious physiological action, but is of especial interest because in the first place of its wide distribution in a variety of vegetable tissues and in the second place because **Nicotinic Acid**, from which it is derived by methylation, occurs in the polishings from rice.

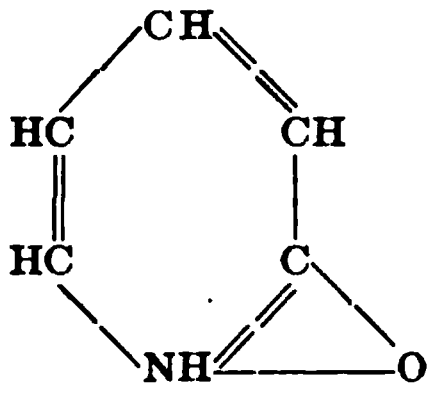
In various parts of the Orient, but particularly Japan and the Philippines, where rice constitutes a very large proportion of the dietary, the introduction of milling methods which involve stripping off the pericarp, or "polishing" of rice has led to the widespread occurrence of a disease known as **Beri-Beri**, the ravages of which were particularly prominent in the Japanese army during the Russo-Japanese War. The disease is evidenced by general lassitude accompanied by anesthesia in certain areas of the skin, edema of the ankles and face, partial paralysis of the leg-muscles and, toward the termination of the disease, distress in breathing. These symptoms are traceable to a widespread peripheral neuritis, beginning in the nerve-fibers most remote from the central nervous system and travelling centripetally. The mortality is very high. It was pointed out by Eijkman in 1897 that beri-beri could be prevented by eating unpolished rice with the pericarp intact, and that it could furthermore be cured by the administration of rice-polishings ("rice-bran"). He discovered that a very similar disease, involving peripheral polyneuritis and ultimate death, could be induced artificially in pigeons by feeding them exclusively upon polished rice. The inference was plain that a preventive and curative substance is present in the pericarp of rice.

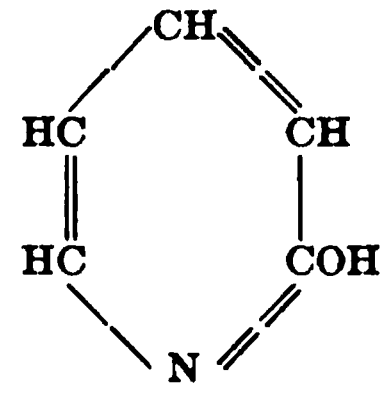
The nature of this substance has been extensively investigated by C. Funk and many others. Funk has succeeded in isolating a curative crystalline substance from **Yeast** which is exceedingly potent, as little as two milligrammes restoring the power of movement within three hours to pigeons which have been completely paralysed by a diet of polished rice. Curative substances are also found in a variety of other foodstuffs and in animal tissues. They are soluble in water and in alcohol, but insoluble, or sparingly soluble in ether. An active curative substance is invariably found to yield a blue color when mixed with Folin and Macallum's "**Uric Acid Reagent,**" which is a solution of sodium phosphotungstate containing a specified proportion of phos-

phoric acid and sodium tungstate. A similar color is yielded by uric acid, alloxantin, dihydrophenylalanine, amino-tyrosine, and certain di- or tri-phenols, but not by purine or pyrimidine bases other than those mentioned, nor by tyrosine itself.

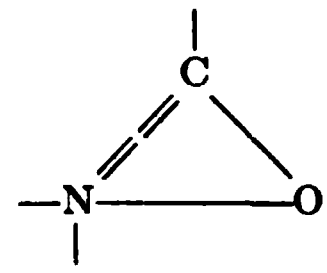
The curative substances isolated by Funk have been termed by him **Vitamines.** From rice-polishings a crystalline curative fraction was obtained which, on fractional crystallization was separated into two substances. The one proved to be **Nicotinic Acid,** which, in the pure crystallized condition, is devoid of curative action, and the other an unidentified nitrogenous substance which tends to lose its curative power with successive purifications. The curative substance from yeast was similarly found to yield nicotinic acid and an unknown nitrogenous base.

From the fact that some of the pyrimidine derivatives have a weak curative action on polyneuritis, it was at first thought that the vitamines were probably pyrimidine derivatives. The more recent investigations of R. R. Williams indicate that the curative principles may be substances having a **Betaine** structure. Thus **α-Hydroxypyridine** has a definitely curative action upon artificially induced polyneuritis, so long as it yields needle-shaped crystals, but these crystals spontaneously change, on standing, into crystalline granules which are quite devoid of antineuritic properties. Now α-hydroxypyridine may conceivably exist in a variety of chemical forms of which the following are examples:





The curative variety, yielding needle-shaped crystals, is probably the pseudobetaine form, resembling the betaines in containing the group:



and the fact that this structure and the antineuritic properties of α-hydroxypyridine spontaneously disappear on standing is suggestive in view of the fact that the curative substances isolated from yeast and rice-polishings by Funk tend also to lose antineuritic power spontaneously on standing or on repeated purification.

The betaines themselves, such as trimethylglycine or trigonellin are impotent to protect pigeons fed on polished rice from the development of polyneuritis. It is, however, characteristic of the betaines that the anhydride ring is very unstable and readily opens up, as, for

example, when salts are formed with acids, and failure to obtain marked curative results with these substances may therefore be attributable to absence of the above ring-structure in the preparations administered.

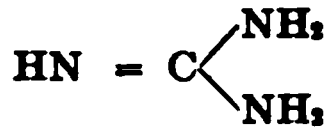
The antineuritic substances in an aqueous extract of yeast may be removed therefrom by shaking up the fluid with fuller's earth. The fuller's earth then becomes "activated" and carries with it all the curative substances. An alkaline extract of this activated fuller's earth was found by Williams and Seidell to exert a marked curative effect, but on recrystallization the substance lost its antineuritic properties and then was identified as **Adenine**. On heating to 180° in sealed tubes with alcohol, a portion of the antineuritic activity was regained, and at the same time it acquired the power, which adenine does not possess, of yielding a blue color with Folin and Macallums' "uric acid reagent." Williams and Seidell infer that the curative substances in this instance is an isomeric modification of adenine.

The instability of the curative substances and the minute proportions in which they are present in antineuritic foodstuffs renders the attainment of any definite conclusions a matter of exceptional difficulty. In the meantime, however, and pending more exact knowledge of this subject, very great care should be taken to avoid confusion by grouping together essentially dissimilar substances of widely differing physiological significance as "vitamines." Such procedure can only lead to mystification, obscures the issue, and obstructs the progress of our knowledge. The term "vitamine" should be definitely restricted to those nitrogenous substances which are known to possess curative action upon **Polyneuritis**. While a variety of other substances are now known to exist, which, in relatively small amounts are essential to health or growth, yet to group them all together as "vitamines" simply deprives the name of its scientific significance. It is much better to use the descriptive term "**Accessory Foodstuffs**," invented by their discoverer, Gowland Hopkins, to include all dietetic factors which are essential constituents of the diet for purposes other than the provision of heat-units or the building-up of carbohydrates, fats and proteins. The hydroxy-acids and other substances in fruits and vegetables which act as **Antiscorbutics** or preventives of scurvy are therefore "accessory foodstuffs" but they are not vitamines. We shall make further reference to the various classes of accessory foodstuffs in later chapters.

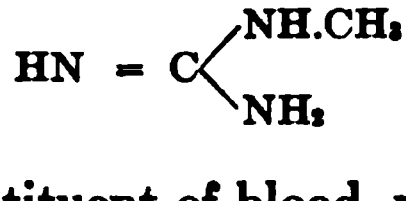
Another deficiency-disease which probably depends upon lack of vitamines, or of substances resembling those which are lacking in polished rice, is **Pellagra**, a condition which is very common in districts such as the Southern United States, where maize furnishes a large proportion of the diet. Milling methods which involve total removal of the pericarp of the grain are believed to be responsible for the disease. Maize deprived of its outer covering has been shown to cause polyneuritis in pigeons in the same way as polished rice.

NITROGENOUS BASES DERIVED FROM GUANIDINE.

Guanidine



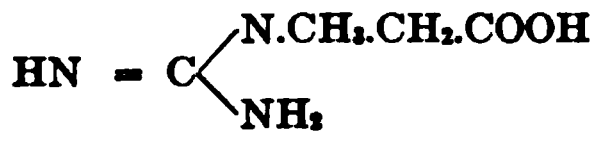
is obtained from proteins by the employment of strong oxidizing reagents, its presence has also been detected in various vegetable tissues, among others in the sugar-beet. It is a strong base, yielding strongly alkaline solutions of very stable salts with acids. It is uncertain whether or not it occurs in traces in the blood and tissues. **Methylguanidine**



is, however, a normal constituent of blood, muscular tissues and urine.

Guanidine and methylguanidine have a very decided physiological action, two hundred milligrammes of methylguanidine being a lethal dose for a guinea-pig. The amount of methylguanidine in the urine is greatly increased by **Anaphylactic Shock**, but the symptoms of poisoning are nowise similar to those of anaphylactic shock. They consist in fibrillar twitchings of the peripheral muscles and an excitation of the spinal cord resembling in comparatively slight measure that produced by strychnine or by **Curare** when directly applied to the cord. In larger doses the myoneural junctions are paralyzed in the same way that they are by curare and the spinal centers are depressed. The fibrillar twitchings produced in muscles by small doses of guanidine or methylguanidine are suppressed by calcium salts and in this respect as well as in the character of the muscular excitation, the action of small doses of guanidine resembles the action of sodium salts upon nerves and muscles.

The marked effect of methylguanidine upon neuromuscular tissues is of especial interest because a derivative of methylguanidine, **Creatine**, or methyl-guanidine-acetic acid:



is the most abundant nitrogenous base in muscular tissues. The percentage of creatine varies in different muscles, being higher in voluntary (striated) than in involuntary (smooth) muscles. In given muscles the percentage of creatine varies in different species of animals, but is remarkably constant in different individuals of the same species. The following are the percentages of creatine found in the muscle of various animals by Myers and Fine.

Species.	Per cent. of creatine.
Rabbit	0.52
Cat	0.45
Man	0.39
Dog	0.37

In the urine the anhydride of creatine, **Creatinine**, is an important constituent:

As a rule creatine itself is not found in mammalian urine, although it replaces creatinine in the urine of birds and is a normal constituent of the urine of young children. In women creatine occurs in the urine immediately after menstruation and occurs in large amounts in the urine during the involution of the uterus which follows delivery. It is considered probable by Folin and others that the creatinine in urine is not derived from the creatine of the muscles but represents a product of the catabolism of protoplasm. Creatine administered by mouth in small doses does not appear in the urine either as such or as creatinine.

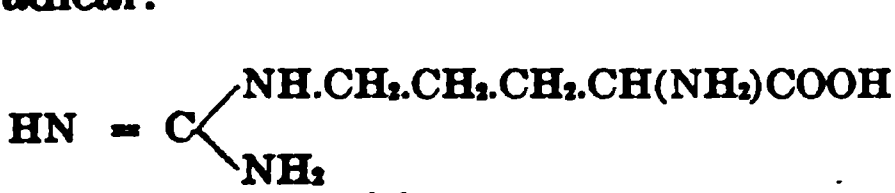
Neither urinary creatinine nor the creatine in muscles is increased by muscular work, but the creatine content of muscles appears to be connected with their **Tonus** or degree of moderate contraction when at rest. Thus standing at "attention" in a military position increases the urinary creatinine while a long march does not. On the other hand if, as much of the evidence seems to indicate, urinary creatinine is not derived from the creatine of muscles but from the "wear and tear", of tissues this result may merely indicate that standing at "attention" involves more destruction of muscular tissues than the performance of muscular work.

Creatine is one of the relatively few substances which stimulate the gray matter or **Neurones** of the cerebral cortex. The customary stimulants for nerve-fibers, calcium-precipitating substances, barium chloride and so forth, have no action upon nerve-cells. Creatine is devoid of stimulating action upon nerve-cells but when applied to the motor areas of the cortex, it throws the animal into convulsions. This may be connected with the fact that the convulsions which accompany **Eclampsia**, a metabolic disease of pregnancy, are heralded by a sharp rise in the creatine output in the urine.

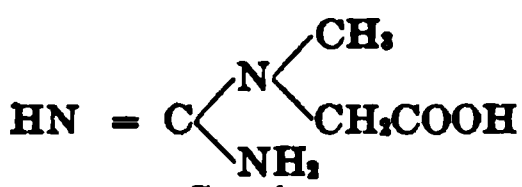
Creatinine may be detected by **Jaffe's Reaction**, which consists in the red color produced by creatinine in alkaline solutions when **Picric Acid** is added. The color is due to **Picramic Acid** which is formed by reduction of picric acid. This reaction is employed for the quantitative estimation of creatinine. Creatinine also gives a ruby-red color with **Sodium Nitroprusside** in alkaline solution (**Weyl's Reaction**).

Creatine may be converted into creatinine by boiling with dilute hydrochloric acid, or it may be determined directly by utilizing the pink coloration which it yields with **Diacetyl**.

It should be noted that creatine is closely related to **Arginine**, which is the only product of protein hydrolysis that contains a guanidine radical:



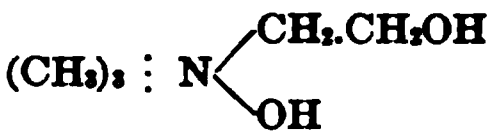
Arginine.



Creatine.

THE NITROGENOUS BASES DERIVED FROM THE PHOSPHOLIPINS.

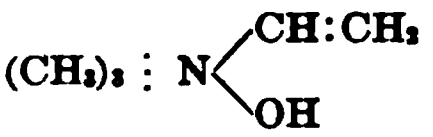
The saponification of the **Lecithins** by alkalies yields, besides soaps and the glycerophosphate of the alkali, a nitrogenous base, **Choline** or trimethyloxyethylammonium hydroxide.



It is a strong base, yielding alkaline solutions and forming a double salt with platinic chloride. By the saponification of **Kephalins**, however, we obtain a different base, namely **Amino-ethyl Alcohol.**

from which choline is probably derived by methylation.

There has been much discussion of the question whether or not a third and related base, **Neurine**, or vinyltrimethyl ammonium hydroxide:



is yielded by the hydrolysis of **Protagon**, but the consensus of opinion appears now to coincide with the view originally expressed by Gulewitsch, that neurine is in reality a putrefaction-product derived from choline by the action of bacteria. Thus perfectly fresh brain-tissue does not appear to yield neurine at all, unless the lecithins (or protagon) are boiled with strong alkalies which, even in pure solutions, results in a partial conversion of choline into neurine.

Both choline and neurine exert the physiological actions which are typical of all the trimethylamine derivatives. The first symptom of poisoning is salivation, followed by intestinal cramps. There is a preliminary fall in blood-pressure succeeded by a rise. Death is ultimately due to arrest of the heart. These symptoms arise from stimulation of sympathetic nerve-endings in the glands or muscles affected and are prevented by the administration of **Atropine** which paralyzes these junctions.

It was at one time thought that free choline might occur in the brain, particularly in degenerative changes of the central nervous system, and that under these conditions choline might be found in the cerebrospinal fluid. The presence of choline in cerebrospinal fluid was, in fact, suggested as a means of detecting degenerative lesions of the brain. Since platinic chloride must be employed to detect the small quantities of choline looked for, however, and potassium and ammonium salts, both of which are also present, yield very similar crystalline platinichlorides, it is rather probable that the crystals obtained from cerebrospinal fluid are not in reality compounds of choline. The

small quantities which are obtainable renders investigation of this question by direct analysis a very difficult one. The free choline alleged to have been detected in brain-tissue has been found to be a postmortem product arising from autolysis or putrefaction.

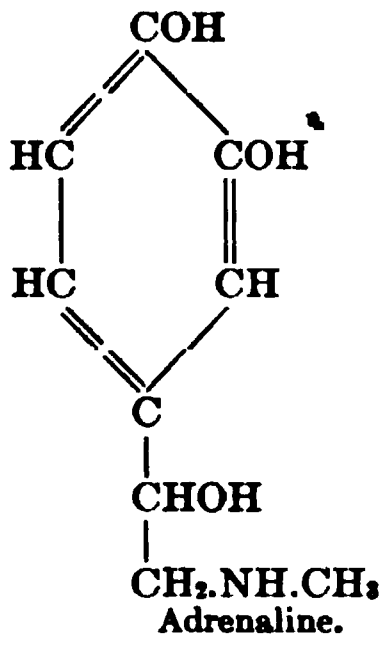
The physiological action of neurine is much more intense than that of choline, being effective in about one twentieth of the dosage. By introducing radicals into the oxyethyl group, however, as in **Acetylcholine** and the nitrous acid or nitric acid esters of choline, substances of very much more intense physiological activity than choline itself are produced.

By the hydrolysis of the **Cerebrosides**, phrenosin and kerasin, a nitrogenous base, **Sphingosine** is obtained the constitution of which is at present unknown. Its percentage composition corresponds to the formula $C_{17}H_{35}NO_2$, and it is a diatomic alcohol containing an amino-group. When sphingosine is heated with concentrated sulphuric acid and a sugar, it yields a purple-violet coloration. The cerebrosides, when similarly treated, first dissolve in the sulphuric acid, yielding a clear yellow solution, and then the sphingosine is split off and separates out in droplets which yield the reaction. The addition of sugar in this case is unnecessary because it is supplied by the galactose in the cerebroside. Regarding the physiological actions of sphingosine nothing definite is known.

NITROGENOUS BASES FORMING THE ACTIVE PRINCIPLES OF INTERNAL SECRETIONS.

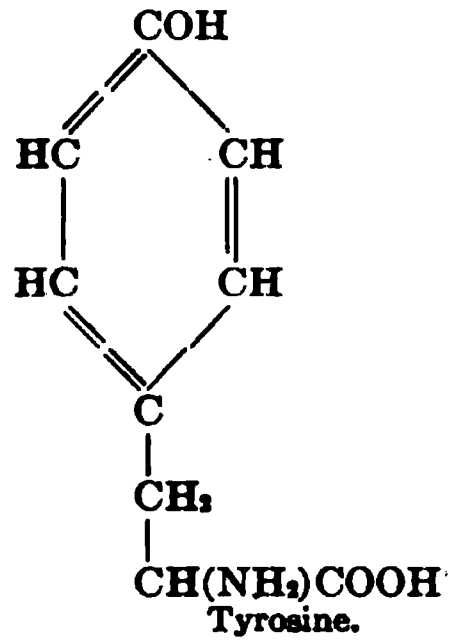
Of these the best studied and, therefore, the best known is **Adrenaline**, the blood-pressure raising or **Pressor** principle of the **Suprarenal Gland**. Other names by which this substance is designated in current literature are **Epinephrin**, **Adrenin** and **Suprarenin**. The term adrenaline is that most customarily used although epinephrin is also frequently employed.

Adrenaline is a derivative of **Catechol** and possesses the following constitutional formula:



Adrenaline.

it is therefore, a methylamine and also a dihydroxybenzene derivative. The structure of adrenaline may be compared with that of tyrosine:



Tyrosine.

from which it will be evident that adrenaline and tyrosine contain the same skeleton of carbon atoms.

The immense physiological importance of the suprarenal gland was first demonstrated by Addison, in 1849, when he showed that the disease now named after him, was connected with degenerative changes of the suprarenal bodies. A few years later it was also discovered that the suprarenal glands contain a "chromogenic substance" which yields a vivid green color with ferric chloride and a red color with iodine. Strangely enough, however, the remarkable effect of suprarenal extracts upon blood-pressure was not discovered until 1894, and the positive identification of the pressor-substances with the "chromogenic substance" was not established until some years later.

Pure adrenaline crystallizes in colorless spherules; it is sparingly soluble in water and almost insoluble in most organic solvents, it will however, dissolve in glacial acetic acid, ethyl oxalate or benzaldehyde.

Adrenaline may be prepared from fresh suprarenal glands by extracting the minced tissue with water, coagulating the proteins by heat or trichloracetic acid, concentrating the extract and adding ammonia which causes the adrenaline to separate out.

The readily oxidizable catechol (orthodihydrobenzol) nucleus in adrenaline is responsible for a variety of color reactions which it yields. The following are among the most characteristic:

Ferric Chloride Reaction.—In neutral or faintly acid solutions adrenaline yields with ferric chloride a vivid grass-green color, which changes to violet, reddish violet and red on rendering the solution alkaline. This will detect about one part of adrenaline in thirty thousand, but the addition of **Sulphanilic Acid**, while changing the color to reddish brown, also renders the test much more sensitive.

Iodine Reaction.—With iodine or iodic acid adrenaline yields a red color. The excess of iodine may be removed by shaking up the mixture with ether.

Mercuric Chloride.—With mercuric chloride, in the presence of **Calcium Salts**, which act as catalyzers, solutions of adrenaline yield a red color.

Persulphate Reaction.—This is the most delicate of all the tests for adrenaline, detecting one part of adrenaline in five million of solution. Potassium persulphate is added to the solution to the extent of one-tenth of a per cent., and the test-tube is then heated by immersion in boiling water.

Phosphotungstic Acid Reaction.—With Folin and Macallum's "uric acid reagent," which consists of a mixture in specified proportions of sodium tungstate and phosphoric acid, adrenaline yields the blue color which **Uric Acid, Alloxantin,** certain **Dihydrophenols, Aminotyrosine** and other substances including the vitamines also yield. The test will detect one part in 3 million of adrenaline.

Adrenaline constitutes about one-tenth of a per cent. of the fresh tissue of the suprarenal gland. One bullock's gland, weighing about ten grams, should therefore yield about ten milligrams.

The origin of adrenaline is unknown. The close relationship to **Tyrosine** would suggest this as the parent substance, but the trans-

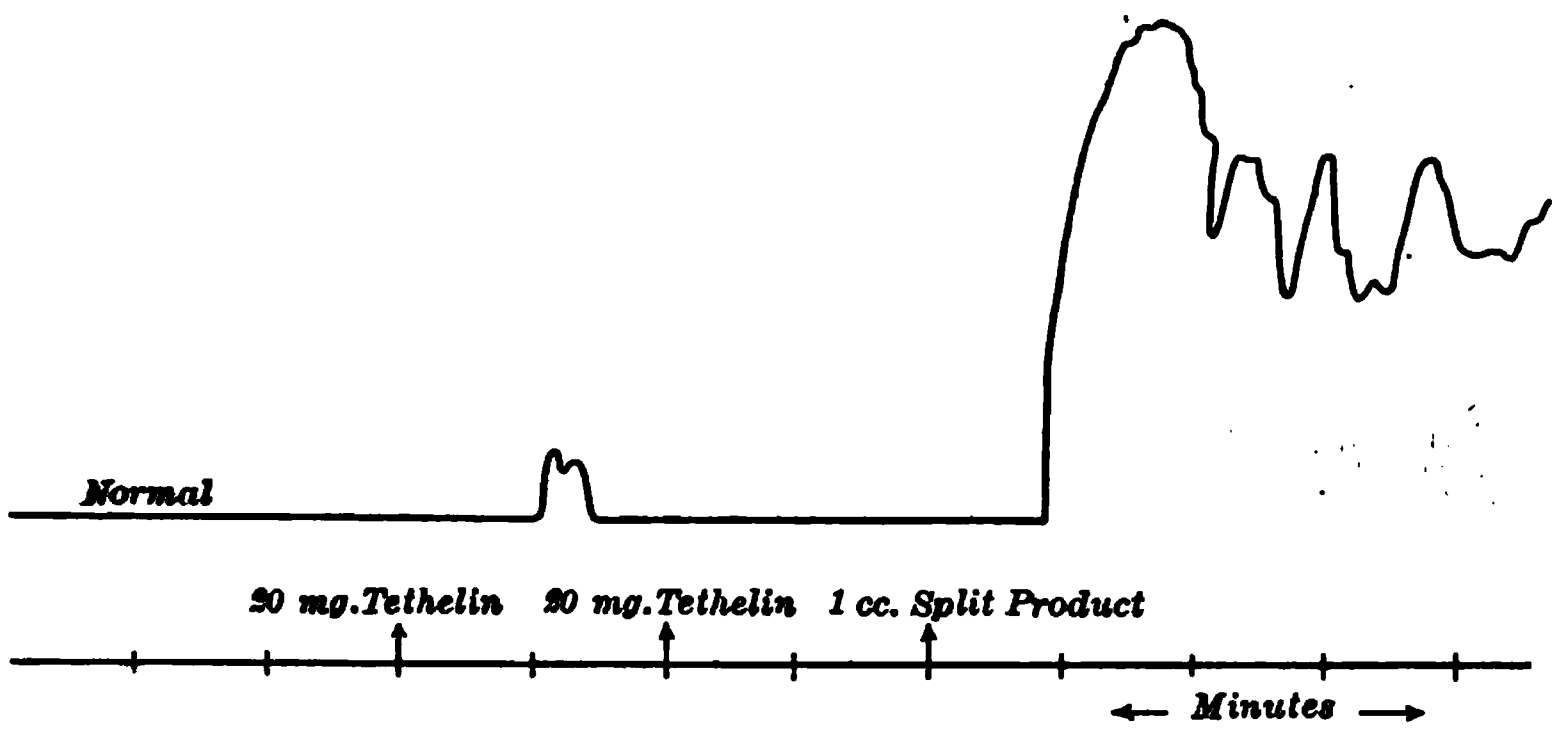


FIG. 6.—Tracing showing effect on the uterus (guinea-pig) of split products of tethelin.

formation of tyrosine into adrenaline would involve a series of changes not merely decarboxylization, but also the introduction of two hydroxyl-groups, one of them in the benzol-ring, followed by methylation of the resultant compound. We are not familiar with any mechanism which could bring about this rather complicated series of transformations. There are some indications, however, that the suprarenal glands may contain precursors of adrenaline which are devoid of pressor-action, and yet yield a coloration with oxidizing-agents.

The important physiological actions of adrenaline will be separately discussed (cf. Chapter XVI).

The posterior lobe or **Infundibulum** of the **Pituitary Body** contains a nitrogenous substance which exerts an action upon the uterus as distinctive as that of ergot and has also the peculiar property of exciting the secretion of the **Mammary Glands.** The structure and even the composition of the active substance are unknown, but since it yields a red color with **Diazobenzene Sulphonic Acid** (see **Histidine**), it probably

contains an iminazolyl radical and is, therefore, related to **Ergamine**. The active substance which, in aqueous solutions, is known by the trade name of **Pituitrin**, gives the **Biuret-reaction** and its activity is rapidly destroyed by trypsin; it is consequently believed to be a **Peptamine** or an amine derived from a polypeptide containing a histidine radical. Several synthetic peptamines have been prepared but their actions have hitherto been found to be much weaker than those of the simpler amines.

A parent-material, which yields pituitrin, or at least a substance resembling pituitrin in its action upon the uterus after hydrolysis by acids or alkalies (Fig. 6), is found in the **Anterior Lobe** or glandular portion of the pituitary body. This substance, which is a water-soluble phospholipin, has been designated **Tethelin**. The histological structure and anatomical relationship of the two parts of the pituitary body are such as to suggest that the anterior lobe furnishes some material to the posterior lobe, and it is therefore, possible that the posterior lobe manufactures pituitrin from tethelin supplied to it by the anterior lobe.

A nitrogenous base of unknown composition appears to be the active principle in acidified aqueous extracts of intestinal mucosa which stimulates the secretion of **Pancreatic Juice** when these extracts are injected intravenously. The substance which is known as **Secretin**, is insoluble in neutral water, soluble in dilute acids, and precipitable by mercuric chloride and by picric acid.

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CHAPTER X.

THE HYDROLYZING ENZYMES.

GENERAL CHARACTERISTICS OF THE ENZYMES.

Disaccharides in aqueous solution are hydrolyzed by mineral acids in accordance with the equation:

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$

Any acid will act upon any disaccharide, but the intensity or **Velocity** of **Hydrolysis** varies somewhat with the nature of the acid and of the disaccharide.

The law which connects the time and the extent of the hydrolysis of cane-sugar by acids was first formulated by Wilhelmy in 1850, who showed that *at every instant the same percentage of the hitherto unchanged sugar is hydrolyzed per second.* Thus, if to begin with we have 100 parts of sugar, and of this 5 parts are hydrolyzed in a given interval of time, we have now 95 parts of unchanged sugar left, and in the succeeding interval five hundredths of this will be hydrolyzed. The transformation, therefore, proceeds in the following manner:

	Number of cane-sugar molecules.	Number of sugar-molecules hydrolyzed.
First interval of time	100.00	$\frac{5}{100} \times 100.00 = 5.00$
Second interval of time	95.00	$\frac{5}{100} \times 95.00 = 4.75$
Third interval of time	90.25	$\frac{5}{100} \times 90.25 = 4.51$
Fourth interval of time	85.74	$\frac{5}{100} \times 85.74 = 4.29$
Fifth interval of time	81.45	$\frac{5}{100} \times 81.45 = 4.07$

In other words, unit-mass of sugar, or one hundred molecules of sugar, always decomposes at the same rate, no matter how much or how little sugar, *i.e.*, how many units or what fraction of a unit, is present in the given solution at the moment. If a gram-molecule of sugar be present, just the same percentage of sugar-molecules will be undergoing transformation per second as when five gram-molecules of sugar are present, but in the latter case the *total* amount of transformation observed per second will be five times as great as in the former.

The rationale of this law, which is known as **Wilhelmy's Law**, may be made clear in the following way: In every instant innumerable collisions are taking place between sugar-molecules and water-molecules. Only a small proportion of these collisions are effective in accomplishing the breaking up of a disaccharide molecule. The **proportion** of effective collisions is, however, the same no matter how many sugar-molecules may chance to be present. We may picture to ourselves, without seeking to employ the analogy too literally, the effective collisions as "head-on" collisions, the ineffective collisions being "glancing." Each sugar-molecule is independent of all the rest, and its chance of achieving an effective collision with a water-molecule is the same as that of all the rest. Suppose every thousandth collision is effective, *i. e.*, one tenth of a per cent. of the total collisions per second. If the solution of sugar be 2-molecular, the total number of collisions per cubic centimeter per second will be twice as great as when the solution is 1-molecular, because there are twice as many molecules of sugar in a given space. In each solution the percentage of effective collisions is the same. Out of one thousand collisions in a 2-molecular solution one will be effective, and out of a thousand collisions in a 1-molecular solution one will also be effective. But as there are twice as many collisions per second in the former as there are in the latter solution, there will also be twice as many *effective* collisions per second, and the amount of sugar transformed in a second, *i. e.*, the **Velocity of Hydrolysis** in the 2-molecular solution must be twice as great as it is in the 1-molecular solution.

This very simple relationship may also be expressed in an algebraical formula:

$$\text{Velocity of hydrolysis} = k(a-x)$$

where (a—x) is the mass of unaltered sugar at any instant, "a" being the initial amount and "x" the quantity which has already undergone hydrolysis at the moment of observation. The constant "k" expresses the constant ratio which, as we have seen, subsists between the mass of unhydrolyzed sugar which is present and the velocity with which hydrolyzed sugar is making its appearance. It is, in fact, the velocity of hydrolysis when $(a-x)=1$, that is, when the mass of unconverted sugar is unity, one gram-molecule, or one gram, or whatever mass we may arbitrarily choose as a unit, provided we measure all the quantities in the equation in the terms of the same unit. Also, and this is very important to notice, the constant "k" is a direct measure of the percentage of *effective* collisions between sugar-molecules and water-molecules, for if the percentage of effective collisions be doubled by any means then the velocity of hydrolysis must obviously be doubled also. In the equation:

$$\text{Velocity} = k(a-x)$$

if the term "velocity" is doubled in magnitude, while (a—x) remains unaltered, then k must have been doubled in magnitude. When the percentage of effective collisions is doubled, therefore, k is doubled, and so forth.

We have hitherto not considered the part which the **Acid** plays in bringing about the hydrolysis. Neutral water only hydrolyses sugar extremely slowly, so slowly that the velocity is negligible in comparison with the velocity of hydrolysis in acid solutions. At the completion of hydrolysis the acid is unaltered and is available for bringing about further and, apparently, unlimited hydrolysis. The acid does not, therefore, communicate any *energy* to the system; it merely increases the **Percentage of Effective Collisions** of molecules of sugar with molecules of water. Such an action is termed a **Catalytic Action**, and the agent which brings about the acceleration, in this instance the acid, is termed a **Catalyser** or **Catalyst.** The **Mechanism of Catalysis** is, in this instance, not perfectly clear, but judging from the analogy afforded by the mode of action of many other catalysts, we may conclude that a compound of the disaccharide with acid is formed and that it is this compound which actually undergoes hydrolysis. In many cases of catalysis such compounds of the catalyst with the substance undergoing decomposition, or **Substrate**, have been isolated and identified, so that we feel justified in assuming that if such compounds are not readily detectable in an instance of catalysis such as that afforded by the hydrolysis of cane-sugar by acids, the reason is that only a minute trace of the compound of the catalyst and the substrate is present in the mixture at any moment

The quantity of the compound of the substrate with the catalyst which is present at any moment in the mixture, must, however small, be proportional to the concentration of the catalyzer and also to the concentration of the substrate, for if two substances A and B combine to form a third AB the quantity of this compound formed must be determined, as the **Guldberg and Waage Mass-law** requires, by the equation:

$$\text{Mass of A} \times \text{mass of B} = \text{constant} \times \text{mass of AB}$$

If, then, the concentration (=mass per unit-volume) of the catalyzer be kept constant, the quantity of the substrate-catalyzer compound, in this instance the compound of cane-sugar with acid, must be directly proportional to the concentration of the still unaltered cane-sugar and decrease as it decreases. Since, for all practical purposes of measurement, it is only the molecules of sugar-acid compound which are undergoing hydrolysis it follows that the velocity of hydrolysis must, if the same proportion of these compound molecules is decomposed in each instant, fall off in direct proportion to the concentration of still unaltered sugar, or in other words the equation:

$$\text{Velocity of hydrolysis} = k(a - x)$$

must hold good for a catalyzed as for an uncatalyzed hydrolysis, so long as the concentration of the catalyzer is kept unaltered.

Since the quantity of the acid-sugar compound is also proportionate to the concentration of the acid, it follows that with varying concentrations of acid the velocity of hydrolysis must also vary directly with the concentration of acid. In other words the value of "k" in the above equation is directly proportional to the concentration of catalyst, or:

$$\text{Velocity of hydrolysis} = kf(a - x)$$

where "f" is the concentration of the catalyzer. In fact so accurately does this relationship obtain that the ratio of the velocity of the hydrolysis of cane-sugar to its concentration is very commonly employed as a means of measuring the quantity of free acid (=hydrogen ions) which is present in a solution.

So much for the hydrolysis of cane-sugar and of other disaccharides by acids; but cane-sugar is also hydrolyzed by an **Enzyme**, to wit, **Invertase**. This enzyme is found in yeasts, in certain moulds and bacteria, in green leaves and young twigs, in some fruits and in germinating grains. In mammalia, it is sometimes found in the human gastric juice, but not in the gastric juice of cows. It is also found in weak concentration in other organs. It may be extracted from yeast-cells with water, provided the cells have previously been subjected to the action of some **Plasmolyzing Agent**, or agent which will break up or enhance the permeability of the limiting membrane of the cell, such as alcohol or ether. The invertase can then be precipitated from its watery solution by alcohol, and this precipitate, in nearly neutral solutions in which hydrolysis would otherwise be excessively slow, rapidly decomposes cane-sugar into its constituent hexoses.

It should be clearly understood that, as in the case of the other enzymes, we possess no clue which enables us to decide whether there is only one or whether there are many invertases. We do not know anything whatever concerning the chemical composition of invertase. Our only means of recognizing this enzyme is by the property which it possesses of hydrolyzing cane-sugar, giving rise to **Glucose** and **Fructose**. Any agent which can be extracted from living tissues which does this and is inactivated by high temperatures we call invertase. It is quite conceivable that many different substances can accomplish this hydrolysis. The activity of an invertase preparation is no guide to its individuality, because in the absence of any knowledge of the chemical properties of invertase, we cannot estimate the purity of any preparation. We can recognize certain impurities, such as phosphates, proteins and so forth in a preparation of invertase, and we can remove some of them, wholly or partially. But when easily recognizable impurities have been removed, we cannot tell whether the residuum is a pure material, that is, a chemical individual, or a mixture of different

substances of which perhaps one is active or, perhaps, many. Could we discover any chemical resembling invertase in its solubilities and in its sensitiveness to temperature and so forth, and possessing the action of invertase, we might be inclined to claim an identity between the two and then analysis would give us a criterion of the purity of any given invertase preparation. But at present we have no such criterion, and we cannot say whether a given preparation of invertase is a single or a multiple preparation, or whether it contains 99 per cent. of invertase and 1 per cent. of impurities or, on the contrary, 1 per cent. of invertase and 99 per cent. of impurities.

In this connection the properties and peculiarities of invertase may be regarded as illustrative of the peculiarities which distinguish nearly all of the enzymes. The same difficulties are encountered in the study of each of the enzymes in turn. It might be imagined that the problem of extracting an enzyme in pure condition from a crude preparation of proved activity could be attacked in a manner analogous to that employed in the original discovery of radium; by employing a variety of precipitants and solvents to fractionate the crude preparation, rejecting the inactive and retaining the active fraction in each successive stage of the process. Unfortunately, however, it is found that almost every chemical procedure which we may employ results in some loss of activity by the enzyme. If a fraction of the original crude preparation be precipitated out from the rest it may be found, for example, to contain an amount of active ferment corresponding to fifty per cent. of the amount which was present in the crude preparation, while the residue, after the separation of the precipitate, may be found to contain none of the ferment whatever. In this way successive processes of purification involve successive losses, until the activity of the preparation ultimately disappears altogether. It is for this reason that the more impure preparations of the various soluble enzymes are usually more active than those preparations which are relatively "pure," *i. e.*, contain a smaller variety of substances.

We have referred to the fact that many different enzymes may be mistakenly regarded as one if they chance to possess a common action. As an illustration of how an enzyme regarded as a single chemical individual may become, with increased acquaintance, recognized as multiple, we may cite the **Trypsins**: enzymes which have this in common—that they hydrolyze proteins and peptones in faintly alkaline solutions. Until recently no means of distinguishing between different trypsins were known, and trypsin was tacitly assumed to be one ferment and only one. Now the investigations of Emil Fischer and of Abderhalden have shown us that there are many trypsins, which differ from one another in the relative ease with which they attack different peptide-linkages.

The enzymes are, as a rule, destroyed, or, at least, inactivated by high temperatures. But this is by no means a rule without exceptions.

Thus several of the oxidizing enzymes, or **Oxidases** regain their activity, lost by heating, when the solution is allowed to stand for some time at ordinary temperatures. According to Gramenetski the same phenomenon may be displayed by certain **Diastases** or starch-splitting enzymes and even in some measure by **Trypsin**. The vegetable **Proteases** or protein-splitting enzymes sometimes withstand higher temperatures than the corresponding enzymes of animal origin and Karl Meyer has drawn attention to the rather extraordinary fact that the **Trypsin** which is produced in culture-media by *Bacillus prodigiosus* will withstand heating for fifteen minutes to 100° C, although it is destroyed within thirty minutes at 56° C. This looks rather as though a trypsin-splitting ferment also existed in the culture-medium for which the optimum temperature is about 56°, and which is destroyed by higher temperatures more rapidly than trypsin itself.

It would, therefore, be very unsafe to infer because a substance does not lose its characteristic activity of some type or other when it is heated that it is therefore not an enzyme. It would be still more unsafe, of course, to infer that it is an enzyme simply because it is "inactivated" by heat. Yet both of these inferences, unfortunately, have not infrequently been made in biological and biochemical investigations. In deciding whether or not a substance or material should be classed as an enzyme we should be guided, rather, by its *quantitative* relationship toward the particular activity which it displays. The enzymes are usually effective in relatively minute concentration. It has been estimated, for example, that a certain **Rennin** or milk-coagulating enzyme preparation will convert no less than 500,000 times its weight of casein into the coagulating form, paracasein, and a preparation of pepsin has been obtained which will hydrolyze to peptones 100,000 times its weight of fibrin. The excessive amount of change which may thus be brought about by relatively minute proportions of enzymes almost compels the assumption that they are not consumed during the progress of the changes which they accelerate, for otherwise the enzyme would probably be "used up" long before so immense a proportion of change had been accomplished. It is, however, impossible at the present stage of our knowledge to submit this supposition to vigorous investigation, because the various hydrolyzing enzymes, at all events, are themselves chemically unstable substances and undergo spontaneous transformation resulting in inactivation on standing in aqueous solution. They are carried down together with any precipitates which may be formed in the digestion-mixture and are not infrequently partially bound by or combined with not only the substrate, but also the products of hydrolysis. Any chemical procedure designed to isolate and recover the enzyme from a digest in which it has been operating would involve loss or impairment of the enzyme even if it had been dissolved in distilled water instead of in a solution of the substrate which it attacks and of the products of its hydrolysis. No

attempt to re-isolate an enzyme after it has acted upon a measured amount of substrate, in order to determine the loss of activity it may or may not have sustained during the reaction, can possibly be successful, therefore, in the present inadequate state of our knowledge and our manipulative technique.

A variety of the hydrolyzing enzymes are not only inactivated, temporarily or permanently by heat, but also by exposure to light and particularly to **Ultraviolet Light.** Solutions of enzymes are also temporarily inactivated by intense agitation of the solution of such a character as to give rise to excessive formation of *foam.* It is then found that the enzyme has become concentrated in the foam and is restored to the solution when the foam subsides. A portion of the enzyme also, under these circumstances, becomes temporarily attached to the surface of the containing vessel. This phenomenon is not peculiar to enzymes, however, for it is exhibited, in greater or less measure, by all those substances which, when dissolved in water, reduce the **Surface-tension** of an air-water interface. For example it is displayed to a striking extent by the various **Saponins** or by **Bile-salts.** (See Chapter XIII.) This liability to become concentrated at liquid surfaces is probably the explanation of the striking tendency, to which reference has already been made, of the various hydrolyzing enzymes to be carried down in association with precipitates which form in their solutions. They are similarly "adsorbed" by such substances as animal charcoal or by insoluble proteins.

THE QUANTITATIVE RELATIONSHIPS IN HYDROLYSIS BY ENZYMES.

We have seen that when cane-sugar is hydrolyzed by acids the relationship between the amount of unaltered sugar in the system and the velocity of change is rather a simple one. The two quantities, the amount of unaltered sugar and the velocity of decomposition, are simply proportional to one another and stand in a constant ratio to one another throughout the reaction. The case is not so simple when the hydrolysis is brought about by **Invertase.** It will be recollected that we regard the rapid hydrolysis of cane-sugar by acids as being due to the formation of a compound between the cane-sugar and the acid, this compound being very easily attacked by water. At any instant the percentage of acid thus combined is almost infinitesimal. The amount of this compound is, as usual in such cases, directly proportional to the concentrations of its components, the acid and the sugar, so that we have:

Concentration of sugar-acid compound = constant × concentration of acid × concentration of sugar.

The velocity of hydrolysis is proportional, at every instant, to the concentration of that portion of the sugar which is actually undergoing hydrolysis, *i. e.*, the sugar-acid compound, and so we have:

$$\text{Velocity of hydrolysis} = \text{constant} \times \text{concentration of sugar-acid compound.}$$

Combining the two equations we find that:

$$\text{Velocity of hydrolysis} = k \times \text{concentration of acid} \times \text{concentration of sugar.}$$

For any given concentration of acid, therefore, since the acid is not consumed at all during the reaction, we have:

$$\text{Velocity of hydrolysis} = k(a - x)$$

where $(a - x)$ is the concentration of unhydrolyzed sugar at any instant.

The case would be very different, however, if more than a trace of the catalyst were combined with the sugar. Suppose, for example, that in the early stages of the reaction, all of the catalyst were combined with the sugar; then, so long as there were enough sugar present to combine with all of the catalyst the amount of the catalyst-sugar compound would always be the same, namely the chemical equivalent of the amount of the catalyst in the mixture. But since this is the only portion of the sugar which hydrolyzes at any measurable rate, the velocity of hydrolysis would in that event be constant.

This will, of course, only hold good so long as the sugar which is still unconverted is sufficient to combine with *all* of the catalyzer. As the reaction proceeds, however, a point will be reached at which the amount of sugar is insufficient to bind all of the catalyzer. After this point in the reaction is reached, the amount of the catalyst bound by the sugar, and therefore the velocity of hydrolysis of the sugar, will become progressively less as the conversion proceeds. In fact it will obviously be equal to the quantity of sugar which is still unconverted, and again we shall have the relationship:

Velocity of hydrolysis = k × concentration of unhydrolyzed sugar for any given proportion of acid present in the mixture.

These complications, as has been implied above, are not observed during the hydrolysis of cane-sugar by acids because the proportion of acid which is at any instant bound by the sugar is so small that it has not as yet been quantitatively estimated. But when **Invertase** is the catalyst instead of acid, we meet with precisely the conditions which we have outlined. When the proportion of sugar to invertase is high, all of the invertase is bound by the sugar, and the portion of the sugar which is thus combined is the only portion which undergoes hydrolysis at a perceptible rate. Under such conditions, for a given concentration of invertase, the rate of hydrolysis of the sugar is constant, while for varying amounts of invertase, the velocity of hydrolysis is proportional to the concentration of invertase employed. Algebraically:

$$\text{Velocity} = kF$$

where "k" is a constant and "F" is the concentration of the invertase. As the proportion of sugar to enzyme falls off, however, a point must be reached at which the remnant of sugar is insufficient to bind all of the invertase. At this stage of the reaction, therefore, or if the proportion of sugar to enzyme is small to begin with, the reaction-velocity begins to fall off as the reaction proceeds, in accordance with the formula:

$$\text{Velocity} = kF(a - x)$$

the numerical value of "k" being different in the two cases because, in the latter case, the "k" includes the equilibrium constant for the reaction:

$$\text{Sugar} + \text{catalyst} \rightleftarrows \text{sugar-catalyst compound.}$$

Turning now to the question of the relationship of the **Quantity of Substrate** hydrolyzed to the **Time of Hydrolysis** it is at once manifest that if the relationship

$$\text{Velocity} = kF$$

holds good, then, the quantity of sugar decomposed in each unit of time being the same, the quantity "x" decomposed after time "t" must be given by the equation:

$$x = kFt$$

In the more general case in which the relationship obtains:

$$\text{Velocity} = kF(a - x)$$

the relationship between the mass of substrate transformed and the time occupied in the transformation may be found by a simple operation of the integral calculus to be:

$$\log_e \frac{a}{a-x} = kFt$$

The following results are illustrative of these two types of relationship:

Invertase on cane-sugar (A. J. Brown) Concentrated Substrate:

Grams of cane sugar per 100 c.c.	Grams of cane-sugar inverted in 60 minutes.	$K = \frac{x}{t}$
40.02	1.076	0.179
29.96	1.235	0.206
19.91	1.355	0.226
9.85	1.355	0.226
4.89	1.230	0.206

Invertase on cane-sugar (A. J. Brown) Dilute Substrate:

Grams of cane-sugar per 100 c.c.	Grams of cane-sugar inverted in 60 minutes.	$10^5 K = \frac{10^5}{t} \log_{10} \frac{a}{a-x}$
(2.00)	(0.308)	(132)
1.00	0.249	219
0.50	0.129	239
0.25	0.060	228

Saliva-Diastase on Starch (A. E. Taylor); a = 0.25 per cent. Starch:

Time in minutes.	$10^6 K = \frac{10^6}{t} \log_{10} \frac{a}{a-x}$
30	490
45	465
60	455
75	470
90	465
120	455
150	460
180	455

Trypsin on d-alanyl-d-alanine (Abderhalden and Koelker):

Time in minutes.	$K = \frac{10^2 x}{t}$
5.0	380
6.5	400
7.5	400
16.0	369
22.0	368
28.0	368
30.0	314
38.0	332

Trypsin on Sodium Caseinate (E. H. Walters):

Time in minutes.	$10^4 K = \frac{10^4}{t} \log_{10} \frac{a}{a-x}$
15	18.
30	16.
45	15.
60	14.
75	13.
90	12.5
105	12.
120	12.
135	12.
150	13.
165	13.
180	12.5
210	13.
240	12.5
270	12.
300	13.5
330	13.
360	14.
420	14.5
480	14.
540	14.

In certain instances yet another type of relationship between the amount of transformation and the time may be observed. In the clotting of milk by **Rennin** and in the hydrolysis of proteins by **Pepsin** we find that the mass "x" of the substance transformed is connected with the time "t," the initial concentration of the substrate "a," and the concentration of the enzyme "F" by the relationship:

$$x = k\sqrt{aFt}$$

which is known as the **Schütz-Borissoff Rule.** The rule only holds good, however, during the earlier stages of the hydrolysis and before about fifty per cent. of the substrate has been hydrolyzed. Arrhenius has found that an exactly similar relationship obtains between the time and the extent of the transformation in the hydrolysis of **Ethyl Acetate** by ammonia. He accounts for it by the fact that in this instance, and presumably also in pepsin-digests, the catalyzer is bound and inactivated by one of the products of the hydrolysis; thus ammonia combines with the acetic acid which is liberated by the hydrolysis of ethyl acetate, forming ammonium acetate which exerts no catalytic action.

If we differentiate the above algebraical expression of the Schütz-Borissoff rule we obtain:

$$\frac{dx}{dt} = \text{velocity of hydrolysis} = \frac{k^2}{2}\frac{aF}{x}$$

from which it is evident that the velocity of hydrolysis is inversely proportional to its extent. We can account for this by supposing that the enzyme combines with a product of the hydrolysis to form an inactive compound according to the equation:

$$\text{Free enzyme} + \text{product} = \text{inactive compound}$$

and that the concentration of enzyme is so small compared with that of the substrate that in the first few moments after digestion has begun the concentration of the inactive compound is nearly equal to the whole of the initial concentration of the enzyme.[1] The trace of free and active enzyme which then remains will be given by:

$$\text{Concentration of free enzyme} = \text{constant} \times \frac{\text{initial concentration of enzyme}}{\text{concentration of product}}$$

or, expressed algebraically:

$$\text{Concentration of free enzyme} = \frac{kF}{x}$$

The actual velocity of hydrolysis must be proportional to the concentration of active catalyst and also to the concentration of unconsumed substrate; hence we have:

$$\text{Velocity of hydrolysis} = \frac{kF}{x} k^1(a - x)$$

where "k^1" is a proportionality-factor which differs in meaning and magnitude from "k."

[1] These conditions obviously do not hold in a mixture of ethyl acetate and ammonia, but the progressive modification of the electrolytic dissociation of the residual ammonia by the ammonium acetate which is formed during the reaction brings about very similar quantitative relationships. The conditions depicted, however, correspond much more closely to those which we may reasonably expect to obtain in a mixture such as that furnished by a pepsin-digest than to those which actually obtain in a mixture of ammonia and ethyl acetate.

The integration of this equation would lead to the relationship

$$\log_e \frac{a}{a - x} - x = kFt$$

which can be shown in many instances to be the relationship which actually does obtain. When x is small, however, that is, in the early stages of the digestion, "a—x" is nearly equal to "a;" the velocity equation becomes:

$$\text{Velocity of hydrolysis} = \frac{kFa}{x}$$

and the integrated expression becomes:

$$x = \sqrt{2kFat}$$

which is the Schütz-Borissoff rule.

The following is an instance of the applicability of these relationships:

DIGESTION OF EGG-ALBUMIN BY PEPSIN FOLLOWED BY THE CHANGES IN THE ELECTRICAL CONDUCTIVITY OF THE MIXTURE (J. SJÖQVIST).

Hours.	Protein digested	$k_a = \frac{\log \frac{a}{a-x} - x}{t}$	$k_a = \frac{x}{\sqrt{t}}$
2	10.5	3.0	7.5
4	16.4	3.8	8.2
6	19.9	3.8	8.1
8	22.7	3.8	8.0
12	27.0	3.7	7.7
16	30.4	3.6	7.6
20	33.7	3.7	7.5
32	40.0	3.4	7.1
48	45.1	3.2	6.5
64	50.8	3.1	6.3
96	57.4	2.8	5.9

Both formulæ give tolerably uniform values for the constants, but those obtained by the complete logarithmic formula are more nearly uniform than those obtained by the employment of the partial expression, the Schütz-Borissoff rule.

Whatever may be the relationship between the extent of transformation and the time which may chance to obtain in a given instance of an enzymatic hydrolysis, one quantitative relationship remains invariably true, namely, that *the time required to attain a given amount of transformation of the substrate is inversely proportional to the concentration of the enzyme*. There appears to be no deviation from this rule which is not immediately explicable by decomposition of the enzyme or such adventitious factors as fluctuation of temperature, reaction and so forth. The following are instances of the applicability of this, the only universal rule which has been found to govern the action of the hydrolyzing enzymes:

TIME REQUIRED TO LIQUEFY GELATIN HARDENED BY THYMOL, WITH VARYING QUANTITIES OF TRYPSIN (MADSEN AND WALBUM.)

F = Concentration of Trypsin.	t = Time required in hours.	Ft.
0.105	0.5	0.052
0.050	1.0	0.050
0.027	2.0	0.054
0.020	3.0	0.060
0.015	4.0	0.060
0.011	5.0	0.055
0.009	6.0	0.054
0.0072	8.0	0.058
0.0060	10.0	0.060
0.0037	16.0	0.059
0.0032	18.0	0.058
0.0027	20.0	0.054
0.0025	22.0	0.055
0.0022	24.0	0.053

COAGULATION OF MILK BY RENNET (MADSEN AND WALBUM).

F = Concentration of Rennet.	t = Time in minutes.	Ft.
8.00	4	32
5.00	6	30
3.30	9	30
1.90	12	23
1.30	20	26
0.70	30	21
0.70	35	25
0.50	50	25
0.40	70	28
0.32	80	26
0.28	100	28
0.25	120	30
0.185	180	33
0.167	200	40

THE INFLUENCE OF TEMPERATURE UPON ENZYMES.

The general effect of increasing temperature upon the hydrolyzing enzymes is to accelerate their action. This favorable influence is, however, limited by the fact that after the temperature exceeds a certain *optimum* the auto-inactivation of the enzyme, which always takes place at a perceptible rate in enzyme-solutions, even at ordinary temperatures, becomes so rapid as to more than counterbalance the acceleration of its hydrolyzing action. The **Optimum Temperature** for enzyme action was formerly supposed to be a characteristic of each enzyme, distinguishing it more or less sharply from other enzymes. We now recognize, however, that while in some measure the optimum temperature does characterize certain groups of enzymes, yet it is greatly influenced by a variety of factors other than the nature of the enzyme itself, such as the reaction (acidity or alkalinity) of the medium in which the enzyme is dissolved; the concentration of the enzyme itself and the nature and concentration of the substrate upon which it is acting. It is, of course, impossible to render all of these conditions

comparable in experiments with different enzymes, and we are therefore left frequently in uncertainty whether the observed differences in temperature-optimum are in reality due to specific differences between the enzymes investigated or are not simply attributable to the circumstances attending their action. In the very great majority of cases, however, it is found that the temperature-optimum from hydrolyzing enzymes lies very slightly above the body-temperature of the warm-blooded animals, namely between 40° and 45° C., the normal temperature of man being 37.8° C. and the temperature of birds about 41° C.

This remarkable correspondence is certainly not accidental, and we may infer that the processes which have brought about the evolution of the warm-blooded animals from cold-blooded ancestors has consisted essentially in an improvement of the adaptation of the more recent forms to the properties of their enzymes, whereby swifter transformations and exchanges of material are rendered possible without at the same time incurring the wasteful expenditure of catalysts which would be involved by still higher bodily temperatures. The factor which determines the bodily temperature of the warm-blooded animals is certainly not the coagulation-temperature of their tissue-proteins, for that lies very considerably above the maximum body-temperature which is observed in any species. The cold-blooded animals and plants, therefore, are handicapped by a disharmony between the properties of their enzymes and the temperature of their tissues. Whether or not this is in some instances compensated for by greater specific activity of their enzymes or by the production of enzymes in greater quantity is a question which the data at present in our possession do not enable us to answer.

In a few exceptional cases the temperature-optimum lies far above the usual level. We have seen that some enzymes, especially certain oxidizing enzymes and the proteolytic enzymes derived from certain bacteria (*Bacillus prodigiosus*, for example) will withstand the temperature of boiling water. Two vegetable proteolytic enzymes, namely the **Papain** from the pawpaw, or fruit of *Carica papaya* and the **Bromelin** in pineapples act best at about 60° C. Generally speaking, the more nearly neutral the solution of the enzyme and the higher the concentration of substrate it contains, the higher is the optimal temperature. It would therefore appear that acids and alkalies accelerate the inactivation of enzymes and that their substrates protect them, probably owing to the fact that they combine with them.

Exposure of an enzyme to moderately high temperatures, for example 60° C. for some hours generally results, not only in loss of hydrolyzing power, but in the acquirement of a power to *inhibit* the very hydrolysis which the active enzyme normally accelerates. This phenomenon has been attributed by Bayliss to the formation of "**Zymoids**" which, he believes, combine with the enzymes from which they are derived to form an inactive compound. In some instances, however, it appears that the retarding influence of heated enzymes arises not so

much from inactivation of the unheated enzyme as in preferential acceleration of the resynthesis of the substrate, thus opposing the reaction which the unheated enzyme accelerates. If this actually occurs then it is evident that a shift in the equilibrium of the reaction:

$$\text{Substrate} + \text{water} \rightleftarrows \text{products of hydrolysis}$$

must be brought about by the heated enzyme, of such a nature as to increase the proportion of the substrate in equilibrium with its products. Such a shift of equilibrium would, of course, necessitate a consumption of energy and a corresponding alteration in the material bringing it about, *i. e.*, in the heated enzyme.

When heated enzymes are allowed to stand in aqueous solution at room-temperatures they may undergo spontaneous **Reactivation.** This phenomenon has been more especially studied in connection with the **Oxidizing Ferments**, but Gramentzki has observed a similar phenomenon in heated solutions of **Diastase, Invertase** and pancreatic **Trypsin.** The following are illustrative data obtained with the commercial starch-splitting enzyme **Taka-diastase**, obtained from *Aspergillus oryzæ*. The enzyme-solution was heated to 95° and then immediately cooled and allowed to stand at room temperatures.

REACTIVATION OF HEAT-INACTIVATED TAKA-DIASTASE (GRAMENTZKI).

Solution tested.	Hydrolyzing power.
Unheated enzyme	12.0
Heated, immediately after cooling	0.6
Heated, twenty-five minutes after cooling	4.2
Heated, seventy-five minutes after cooling	5.5
Heated, six hours after cooling	8.2
Heated, five days after cooling	12.0

The accelerative influence of raising the temperature upon the hydrolysis of the substrate by an enzyme has been frequently investigated quantitatively, and it has been found that the relationship between the temperature and the velocity of hydrolysis is that which commonly pertains in chemical reactions. It may be expressed as follows:

$$\frac{v_1}{v_0} = e^{\frac{\mu}{2}\left(\frac{t_1 - t_0}{t_1 t_0}\right)}$$

where "v_1" is the velocity of the hydrolysis at the temperature "t_1;" "v_0" is the velocity of hydrolysis at the temperature "t_0;" "e" is the base of the natural or "Napierian" logarithms (2.71828 . . . . .) and "μ" is a constant which is characteristic for the specific reaction, and is expressive of the degree of effect which temperature exerts upon it. The temperature is measured in "absolute" units, that is to say in degrees centigrade above zero *plus* 273°. The relationship only holds good, however, so long as the temperatures employed do not

exceed the "temperature-optimum," otherwise the secondary inactivation of the enzyme introduces a disturbing factor.

It is more simple, however, although less accurate, to estimate the effect of temperature by the change in velocity produced by a rise of 10° C. It is found, as a very general rule, excepting in the case of photochemical reactions, that the value of μ for chemical transformations is of such a magnitude (10,000 or over) that a rise of 10° at ordinary room- or incubator-temperatures doubles or more than doubles the velocity of transformation.

The "**Temperature-coefficient,**" or ratio:

$$\frac{\text{Velocity at T} \quad + \quad 10°}{\text{Velocity at T°}}$$

for chemical reactions is therefore 2 or over, while for purely physical processes, such as changes in viscosity or capillarity or for photochemical reactions the value of the coefficient generally only slightly exceeds unity or, in the case of capillary phenomena, may be less than unity.

The following are illustrative values of "μ" for various hydrolyses brought about by enzymes. For comparison the value of μ for the hydrolysis of cane-sugar by acids is included.

Process.	μ
Hydrolysis of cane-sugar by acids	25,600
Hydrolysis of cane-sugar by invertase	11,000
Hydrolysis of starch by amylase	12,300
Hydrolysis of triacetin by lipase	16,700
Hydrolysis of egg-albumin by pepsin	15,570
Hydrolysis of casein by trypsin	37,500
Inactivation of rennet	90,000
Inactivation of pepsin	75,000
Inactivation of invertase	72,000
Inactivation of trypsin	62,000

On comparing these various figures it will be seen that the effect of temperature upon enzymatic hydrolyses is of the same general order as its effect upon other chemical reactions. The **Inactivation** of an enzyme by heat, however, is exceptionally accelerated by rise of temperature, the coefficients for all inactivations being very much higher than those for the hydrolyses which the enzymes accelerate. This accounts for the relative "steepness" with which the curve of enzymatic activity falls off after the temperature has passed the optimum; at this point the inactivation of the enzyme is very much accelerated by a rise in temperature sufficient only to produce a slight modification of the velocity of the hydrolysis which the enzyme is accomplishing.

Enzymes are also inactivated by exposure of their solutions to **Light,** and especially to the **Ultra-violet Rays.** The inactivation by ultra-violet light occurs in the absence of oxygen, but the visible rays of light, especially in the presence of fluorescent dyes such as **Eosin,** are

also able to inactivate enzymes provided oxygen be present. Evidently two different types of change in enzymes may be brought about by light, involving different parts of the spectrum.

THE INFLUENCE OF REACTION UPON HYDROLYSES BY ENZYMES.

The great majority of the enzymes are very decidedly influenced by the reaction, or H^+ or OH^- ion concentration of the medium in which they act. For each enzyme or group of enzymes there is a certain range of H^+ or OH^- concentrations within which they work best and below or above which their activity is impeded. The upper limit of H^+ or OH^- concentrations is set by the destruction of the enzyme which rapidly occurs in solutions which are too acid or alkaline. The factor which sets the lower limit is not so easy to perceive since the stability of the enzymes in neutral solutions is often greater than it is in the faintly acid or alkaline solutions in which their hydrolyzing activity is most favorably displayed.

The most striking dependence upon reaction is shown by the **Proteolytic Enzymes, Pepsin** and **Trypsin.** Pepsin acts best in a faintly acid, while trypsin acts best in a faintly alkaline medium. A slight excess of alkali rapidly destroys pepsin, while an even slighter acidity inactivates trypsin. Both of these enzymes will hydrolyze proteins in neutral solutions, but their activity is much inferior to that which they will display in a medium of favorable reaction.

So far as pepsin is concerned it is not difficult to infer that the need for a slight acidity of the medium arises from the fact that a compound of the pepsin with the acid is formed which possesses much greater proteolytic power than the uncombined pepsin. This is evidenced by the fact that not all acids are equally efficient in promoting the hydrolysis of protein by pepsin, there being a marked specificity in the relationship of pepsin to **Hydrochloric Acid.** While other acids will accelerate the hydrolysis of proteins by pepsin, their accelerative influence is far inferior to that of hydrochloric acid, and the favoring action of different acids, instead of running parallel to their degree of electrolytic dissociation, as we should expect if it were an effect purely due to hydrogen ions, bears, in fact, no relationship to the "strength," *i. e.*, dissociation of the acid. **Lactic Acid**, for example, in equimolecular solutions, has a more favorable effect than sulphuric acid.

In the case of **Trypsin** the accelerative action of alkalies runs strictly parallel to their dissociation, so that here we are left in doubt as to whether the effect is due to the formation of a compound of the trypsin with the base employed, or whether the alkali does not, on the contrary, act as an accessory catalyzer, so altering the substrate as to render it more susceptible to attack by the enzyme.

There are certain observations, however, which seem to show that in this case also a compound is formed of the trypsin and the added

base which exerts a much more intense proteolytic action than trypsin itself. For if we follow the hydrolysis by trypsin, of an alkaline solution of casein by means of the gas-chain (potentiometer) so that we obtain a measure of the changes in the actual hydroxyl ion concentration as the hydrolysis proceeds, we find that the alkalinity of the digest progressively diminishes at a uniform rate corresponding to the formula:

$$\text{Velocity} = k(a - x)$$

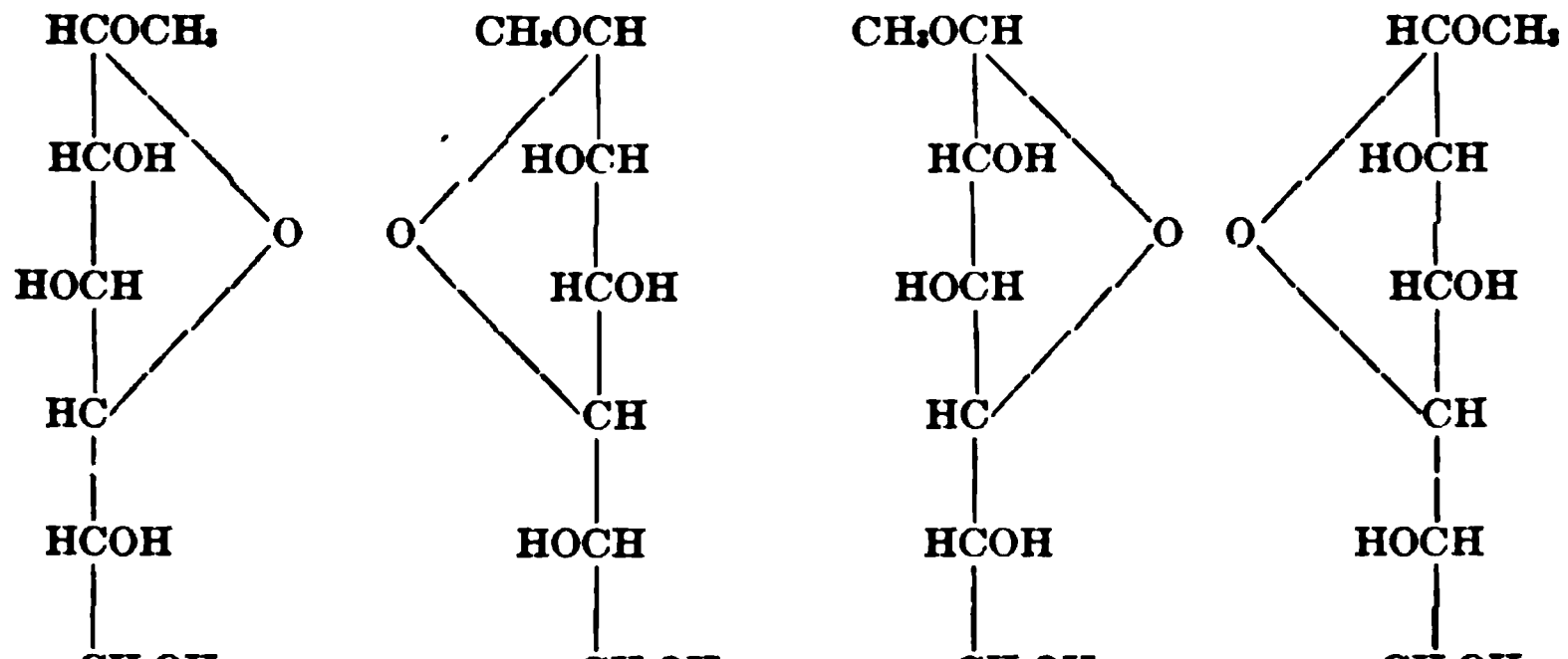
until a certain **Critical Alkalinity** is reached, which lies in the neighborhood of 10^{-6}N or $\frac{N}{1{,}000{,}000}$, below which the velocity of hydrolysis diminishes very much more rapidly than Wilhelmy's law would indicate. For a given concentration of trypsin the critical reaction is exactly the same when a basic protein such as protamine is employed as when the acid protein, casein, is the substrate. Evidently, therefore, this sudden falling off in the velocity of hydrolysis is not due to any relationship of the **Substrate** to the free alkali in the digest, but rather to a relationship of the **Enzyme** to the free alkali. The result is, in fact, exactly what one would expect to obtain if the actual catalyst were a compound of trypsin with the alkali. The concentration of the catalyst would remain constant at all alkalinities below those destructive of the enzyme, provided there was a sufficient amount of unneutralized alkali present to combine with all of the trypsin. Directly the concentration of free alkali fell below this limit, however, the concentration of active enzyme would diminish in proportion to the diminution of alkalinity, that is to say, in proportion to the extent of hydrolysis, and the velocity of hydrolysis would fall off correspondingly rapidly.

Since in these two instances we have experimental ground for the belief that the favorable influence of dilute acids or alkalies upon the activity of the enzyme is due to the formation of compounds with the enzyme, we may infer that the mechanism of the acceleration by acids or alkalies is probably the same in other cases.

THE SPECIFICITY OF THE HYDROLYZING ENZYMES.

The various enzymes which hydrolyze **Disaccharides** and **Glucosides** are highly *specific* in their action, that is to say, a given enzyme will hydrolyze a particular disaccharide or a particular type of glucoside and no other. A very beautiful example of the specific relationship which subsists between the structure of a glucoside and the nature of the enzyme which attacks it is that afforded by the enzymatic hydrolysis of the various **Methyl Glucosides.** Four of these glucosides are known, namely α-methyl-l-glucoside, and β-methyl-l-glucoside,

α-methyl-d-glucoside and β-methyl-d-glucoside. Their structures are represented below:



α-methyl-d-glucoside. | α-methyl-l-glucoside. | β-methyl-d-glucoside. | β-methyl-l-glucoside.

Of these neither α- nor β-methyl-l-glucoside are acted upon by enzymes. The α-methyl-d-glucoside is hydrolyzed by the **Maltase** in yeast, but the β-methyl-d-glucoside is not hydrolyzed by yeast; it is, on the other hand, hydrolyzed by the enzyme **Emulsin** which is found in the kernels of stony fruits such as the almond and in the tissues of the fungus *Aspergillus niger*. But emulsin is without action on the α-methyl-d-glucoside.

Similarly, **Invertase**, which hydrolyzes cane-sugar to glucose and fructose, will not hydrolyze maltose; maltase, which hydrolyzes maltose to two molecules of glucose, will not attack cane-sugar or lactose; lactase, which hydrolyzes milk-sugar, will not hydrolyze maltose or cane-sugar.

Since these various disaccharides differ from one another only in the arrangement of the various groups about the central carbon-skeleton, the high degree of specific interrelationship with the enzymes which attack them which they display, led Emil Fischer to the view frequently alluded to as the **Lock-and-key Hypothesis**, whereby the enzyme is supposed to possess a structure which fits a particular disaccharide or glucoside as the grooves of a key fit the wards of a lock. Indeed there is no other way in which we can imagine a mechanism which will so precisely pick out a particular arrangement of atoms and decompose that one and no other. The phenomenon affords, in fact, a striking confirmation of the view that these enzymes accomplish the hydrolysis of the disaccharides through the formation of intermediate compounds.

The fat-splitting ferments, or **Lipases** do not exhibit such extremely preferential specificity. Nevertheless some measure of specificity is displayed in certain instances. Thus Dakin found that in a mixture of the menthyl esters of d- and l-**Mandelic Acid** the menthyl-d-mandelate is hydrolyzed by pancreas-lipase much more rapidly than the menthyl-l-mandelate, so that the mandelic acid which results from the hydrolysis is strongly dextrorotatory.

Among the **Proteolytic Enzymes** we again meet with a series of specific relationships between the enzymes and the substances which they hydrolyze. This specificity is not revealed when we act upon **Proteins** with various **Trypsins** since every protein which is soluble contains some of the linkages which are susceptible to attack by any given trypsin. Digestion of a protein will therefore proceed with trypsin from any source, and if the linkages in the molecule which are attacked differ with the type of trypsin employed, we have at present no certain means of ascertaining that fact in so complicated a molecule as that of a protein. It was for this reason that the specificity of different trypsins was until recently totally unsuspected, and it was tacitly assumed that all of the enzymes which hydrolyze native proteins to amino-acids in faintly alkaline solutions were identical. The employment of synthetic **Peptides** of known structure and configuration as substrates, however, has recently revealed to us a heretofore unsuspected multiplicity of protein-hydrolyzing enzymes.

The trypsin in pancreatic juice was found by Fischer and Abderhalden to hydrolyze certain synthetic peptides while others remain unattacked. The various peptides which they investigated were distributed between these two classes as follows:

HYDROLYZED.

*Alanyl-glycine
*Alanyl-alanine
*Leucyl-isoserine
Glycl-l-tyrosine
Leucyl-l-tyrosine
*Alanyl-glycyl-glycine
*Leucyl-glycyl-glycine
*Glycyl-leucyl-alanine
*Alanyl-leucyl glycine
Dialanyl-cystine
Dileucyl-cystine
Tetraglycl-glycine
Triglycl-glycine ester
d-alanyl-d-alanine
d-alanyl-l-leucine
l-leucyl-l-leucine
l-leucyl-d-glutamic acid

NOT HYDROLYZED.

Glycyl-alanine
Glycyl-glycine
Leucyl-alanine
Leucyl-glycine
Aminobutyryl-glycine
Valyl-glycine
Glycyl-phenylalanine
Leucyl-proline
Diglycyl-glycine
Triglycyl-glycine
Dileucylglycyl-glycine
d-alanyl-l-alanine
l-alanyl-d-alanine
l-leucyl-d-leucine
d-leucyl-l-leucine

It will be observed that especially among the various dipeptides formed by the union of d- and l-alanine with d- and l-leucine the specificity of the enzyme is very strongly marked. It will be noted also that mere *length* of the peptide-chain confers upon it susceptibility to attack. Thus diglycyl-glycine and triglycyl-glycine were not attacked, while tetraglycylglycine was hydrolyzed. The compounds marked with an asterisk were racemic, and in every case only one of the optical antipodes was attacked, in every case also the isomer which was hydrolyzed was that which occurs in the native proteins.

The trypsin which is contained in red blood-corpuscles was found to hydrolyze glycyl-l-tyrosine, in this respect resembling the trypsin of pancreatic juice. It also hydrolyzed diglycyl-glycine, however, and therefore it cannot be identical with the trypsin of pancreatic juice. Blood-serum will not hydrolyze glycyl-l-tyrosine and the trypsin which it contains therefore differs both from pancreatic trypsin and red-blood-corpuscle trypsin, yet it will hydrolyze d-l-alanyl-glycine, diglycyl-glycine and tri-glycyl-glycine. The existence of three different trypsins is thus demonstrated, and from these and similar experiments we can infer that the variety of animal trypsins is very great and possibly coextensive with the number of different types of tissue which may comprise the body of a multicellular animal.

THE SYNTHETIC ACTION OF HYDROLYZING ENZYMES.

When we hydrolyze such a substance as **Ethyl Butyrate** with the aid of a non-enzymatic catalyzer, we find that the transformation into ethyl alcohol and butyric acid is never complete, but stops short when, in dilute solutions, about two-thirds of the ester is decomposed. No matter what catalyzer we may employ, or if we allow spontaneous hydrolysis to occur, or bring about hydrolysis by means of a fat-splitting enzyme, the transformation comes to a standstill when about one-third of the ester still remains undecomposed.[1] On the other hand, if we mix ethyl alcohol and buytric acid, and by the agency of catalyzers or otherwise, bring about their combination, we will find that here also the transformation is never complete, but that it comes to a standstill when about one-third of the alcohol and buytric acid have combined to form the ester. Evidently, therefore, from whichever end of the process we start we reach a mixture of the same composition. We cannot suppose that either reaction has then ceased to occur, but we can readily see that in the mixture which no longer alters in composition and is at equilibrium *the forward and reverse actions are proceeding at the same rate:*

$$C_3H_7COOC_2H_5 + H_2O \rightleftarrows C_3H_7COOH + C_2H_5OH$$

In a variety of hydrolyses the same phenomenon is observed, but in the majority of instances the station of equilibrium lies further to the right or left than in the instance chosen for illustration. Thus in the hydrolysis of cane-sugar it lies so far to the left that at equilibrium the hydrolysis is, so far as all practicable measurements are concerned, absolutely complete.

Now a true catalyzer is, as we have seen, not consumed at all during the progress of the process which it accelerates, and, this being the case, it cannot communicate any **Energy** to the system. Any shift of equilibrium in a chemical reaction which absorbs or liberates heat must involve the consumption or absorption of a quantity of energy equivalent to the heat of reaction. But equilibrium, as we have seen,

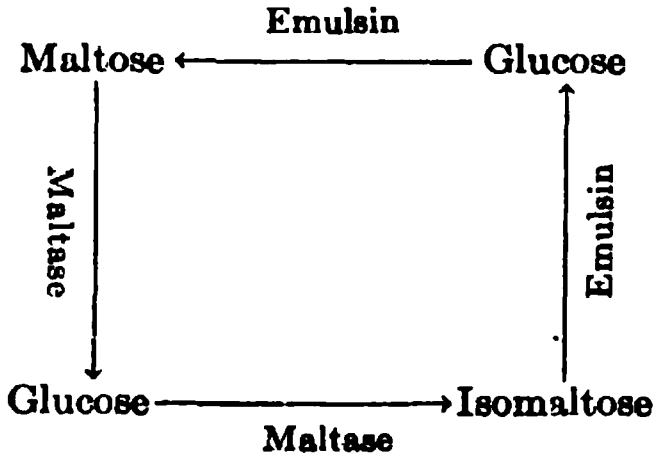
[1] The exact proportion depends, as we shall see, upon the dilution of the solution, *i. e.*, upon the mass of water in the reacting mixture.

occurs only when the forward and reverse velocities are equal, and hence if the forward reaction, or reaction of hydrolysis, is accelerated by a catalyzer, the reverse reaction or reaction of synthesis must also be accelerated and to an exactly equal degree. If, then, the hydrolyzing enzymes are analogous to other catalyzers and are not consumed during the progress of the reactions which they affect, they must accelerate the resynthesis of the substrate from its products no less than the hydrolysis of the substrate itself.

The prediction, based upon the above premises, made by the Dutch chemist Van't Hoff in 1898 that enzymatic syntheses might prove possible was verified experimentally in the same year by Croft Hill, who succeeded in this manner in synthesizing a disaccharide by acting upon a highly concentrated solution of glucose with the enzyme **Maltase** obtained from yeast.

The synthetic disaccharide was, very naturally, assumed to be Maltose, but further investigation showed that the prediction of Van't Hoff had not been so completely verified as was at first supposed, for Emmerling in 1901 showed that the disaccharide which was actually produced in Croft Hill's experiment was not maltose, or glucose-α-glucoside, but a disaccharide which yields a predominating proportion of β-glucose on hydrolysis, namely glucose-β-glucoside, or **Isomaltose.** Now isomaltose is not hydrolyzable by maltase, so that the synthetic activity of the enzyme, instead of reversing the reaction of hydrolysis, produces a disaccharide which it cannot hydrolyze. It would appear that a shift of equilibrium is actually occasioned by the enzyme, but as there is no difference of energy-content between optical isomers, the shift in the station of equilibrium caused by the enzyme is not, so far as the production of isomaltose instead of maltose is concerned, of such a character as to require consumption of the enzyme to accomplish the liberation or absorption of energy.

Isomaltose is, however, hydrolyzable by the enzyme **Emulsin** which occurs in different situations from those in which maltose is found. It became at once a matter of great interest to ascertain what synthetic products would result from the action of emulsin upon concentrated solutions of glucose. This experiment was carried out by E. F. Armstrong, who found that the product resulting from the synthetic action of emulsin was not isomaltose, but **Maltose.** Each enzyme, therefore, synthesiezs that enzyme which it cannot hydrolyze. These relationships may be schematically represented by the following diagram:



Similarly the **Lactase** in kephir yeast was found to synthesize, not **Lactose** which it hydrolyzes, but **Isolactose**, which it does not hydrolyze.

Since the publication of Croft Hill's fundamental observation a great number of enzymatic syntheses have been accomplished. Among carbohydrates, substances resembling **Starch** and **Glycogen** have been synthesized through the action of **Diastases**, while **Triacetyl Glucose** has been formed from acetic acid and glucose under the influence of pancreas-extract. Among the fats **Ethyl Butyrate** has been synthesized from ethyl alcohol and butyric acid, glyceryl butyrate, amyl butyrate, methyl oleate, glyceryl triacetate and glyceryl trioleate have all been synthesized from their components through the reversed action of various **Lipases.** In the case of methyl oleate it has been shown that the pancreas-lipase employed to bring about its synthesis definitely does not affect the final equilibrium which is attained, for the proportion of ester formed after a sufficient lapse of time is independent of the quantity of enzyme employed, only the **Speed** with which the equilibrium is attained being affected. The following are illustrative data:

SYNTHESIS OF METHYL OLEATE (POTTEVIN).

Quantity of pancreas-extract employed.	Percentage of ester formed.		
	1 day.	2 days.	20 days.
1	8	56	84
2	12	66	82
5	21	66	84
10	43	74	85

Quantitative data of this description are very important because the whole question whether the enzymes act as true catalyzers or, on the contrary, enter into and affect the equilibria of the reactions which they accelerate, turns upon the question whether the final proportion of substrate to products is at all influenced by the presence of the enzyme. The results of Pottevin indicate that there is no such influence in the case of pancreas-lipase synthesizing methyl oleate, because if there were, then doubling the amount of enzyme should double the displacement of equilibrium and since no measurable effect upon the equilibrium results from doubling or even multiplying by ten the quantity of enzyme employed, it follows that the single unit of enzyme also did not affect the station of equilibrium. Similarly A. E. Taylor has shown that the station of equilibrium in the hydrolysis of glyceryl triacetate by lipase is exactly the same as that which is obtained when sulphuric is used as the catalyzer. Such measurements are, however, usually lacking in enzyme studies, frequently because of the technical difficulty of measurements extending over the long periods required to attain final equilibrium.

In the case of **Glyceryl Trioleate**, which differs from the glyceride studied by Taylor in being insoluble in water, H. C. Bradley has attained results which point rather clearly toward a decided displacement of equilibrium by the enzyme, for he finds it impossible to procure

any appreciable synthesis of **Triolein** from oleic acid and glycerol in the presence of fifty per cent. of water, although when we start with triolein in this proportion of water an appreciable amount of triolein remains unhydrolyzed at the end of prolonged hydrolysis. The presence of the enzyme in this case appears to selectively accelerate hydrolysis, and if this is the case then the enzyme must be consumed in the process.

Among the proteins the synthesis of a protamine, **Salmine**, from a concentrated solution of its digestion-products has been accomplished by A. E. Taylor, who employed an unusually stable **Trypsin** obtained by extracting the liver of a mollusc (*Schizothærus nuttalli*) with glycerol, and adding this in very large amounts to a saturated solution of the amino-acids which finally result from the hydrolysis of protamine. Only a small proportion, about one-half of a per cent., of the original protein was recovered and that only after a lapse of five months. The identity of the synthetic protein with salmine was deduced from analysis and general physical behavior.

When **Sodium Caseinate** in neutral solution is subjected to hydrolysis by **Pepsin** a group of infraproteins results, which are collectively termed **Paranucleins**, and which subsequently undergo further hydrolysis, with the production of proteoses and peptones. The paranucleins resemble casein in being soluble in dilute alkalies and precipitable by acetic acid, but are less soluble in dilute mineral acids than casein itself. When to the concentrated solution obtained by evaporating down the final products of the prolonged peptic hydrolysis of casein, a very large proportion of fresh pepsin is added, after a comparatively brief period (forty-eight hours) at 40° C. a precipitate is formed in the mixture which appears to be identical with paranuclein. In this case the identity has been confirmed by immunological methods. The antiserum to casein or paranuclein produced by repeatedly injecting these substances into the circulation of rabbits yields no precipitate either with the products of the complete peptic hydrolysis of casein or with pepsin, but it does yield a precipitate with the synthetic paranuclein obtained in the manner outlined, and this precipitate binds the antibodies to casein and paranuclein which the serum contained. Since the **Antibodies** which appear in the circulation of animals as a result of immunizing them against proteins are in the highest degree specific, yielding precipitates only with the protein employed in the immunization or with infraprotein derived from it by partial hydrolysis the synthetic product may be considered to be thus clearly identified with the infraproteins which are the first cleavage-products of casein.

In this case, however, rather definite indications were obtained that the **Synthesizing Enzyme** is not identical with pepsin itself, for it proved possible to bring about the synthesis a great deal more rapidly at 70° C. than at 40° C., while the *hydrolyzing* activity of pepsin is inhibited altogether at this temperature. Moreover not every preparation of pepsin will yield the synthesis. It has been suggested that the active

agent in accomplishing the synthesis is a modification of pepsin, arising from it by loss of water:

$$\text{Hydrolyzing Pepsin} \rightleftarrows \text{Synthesizing Pepsin} + H_2O$$

and that in bringing about the hydrolysis of protein the hydrolyzing form may partially lose water and be transformed into the synthesizing form and *vice versa*, high temperatures, as usual, favoring the formation of the anhydride or synthesizing form. If this were so, of course, pepsin would be far from being a true catalyzer, since it would enter into and be modified by the reactions which it accelerates. Such modification has, however, not yet been shown to occur.

It will be seen, therefore, that while in some instances the hydrolyzing enzymes appear to act as genuine catalyzers, in other instances their behavior appears to be inconsistent with this view. In any case, the synthetic activity of the hydrolyzing enzymes, so far as it has yet been demonstrable *in vitro* is very inferior in point of speed and completeness to the synthetic processes which actually and continually occur in living tissues. As we shall see, glucose is converted into glycogen almost as rapidly as it can be absorbed and transported, dissolved in minute concentration in the blood, to the cells of the liver. Fat is synthesized from glycerol and fatty acids in the intestinal mucosa within a few moments after absorption, there are strong reasons for the belief that the synthesis of protein from amino-acids in the tissues is not much less rapid. The contrast between these phenomena and the prolonged periods of action and high concentrations both of the enzyme and the products required to resynthesize the substrate by a hydrolyzing enzyme, and the fragmentary yield which results, point very strongly to the existence, in living tissues of a decisively different synthesizing mechanism to that involved in the reversed action of catalyzers. Only two alternatives appear to be open to us in interpreting this disproportion. Either the tissues employ enzymes which selectively accelerate syntheses and therefore are consumed by the syntheses which they accomplish, or else the syntheses in living tissues, composed though they are of over eighty per cent. of water, take place under conditions approximating to almost complete desiccation. This latter alternative is not so inconceivable as it might appear, because the reactions in question may possibly take place at the surface of lipoid granules which are emulsified in the protoplasm, but which, being insoluble in water, afford a medium which is almost water-free. The most serious difficulty attaching to this view, however, is that so many of the products so rapidly synthesized in living tissues are as insoluble in oils as water is itself, for example the proteins. On the other hand if the former alternative be adopted we are faced with the difficulty that the hypothetical synthesizing enzymes have never been obtained apart from living tissue, so that either their action is intimately bound up with the uninjured **Structure** of the protoplasm, or else we have not yet hit upon the right methods of extracting them from living tissues and conserving their synthetic activity.

ANTIENZYMES.

Like the proteins and the poisonous products of bacterial metabolism, the various enzymes, when injected into the circulation of living organisms, give rise to specific **Antibodies**, or substances in the circulation of the immunized animal which combine with the enzyme which has been injected.

The **Antienzymes** thus produced are highly specific and bind only the enzyme employed for immunization. Normal blood, however, contains appreciable amounts of antitrypsin and also of antirennet, which latter, however, is stated not to be identical with the antirennet produced by immunization. The antienzymes appear as a rule to be very resistant to heat, withstanding for some time a temperature of 70° without losing their power of inhibiting digestion of the enzymes which they bind.

Antipepsin and **Antitrypsin** also occur in notable quantities in the tissues of **Intestinal Worms**, and it is to this that their immunity to digestion is attributed. The immunity of the tissues of the stomach to digestion by the gastric juice which they produce, and of the tissues of the intestine to digestion by pancreatic juice is similarly attributed to the normal presence, in these tissues, of antienzymes.

The antigenic property of the enzymes rather strongly points toward their ultimate protein nature, for up to the present no substance has been found to produce antibodies on injection which has not been a protein, or a substance possibly contaminated by a protein.

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CHAPTER XI.

THE DIGESTION AND ASSIMILATION OF THE FOODSTUFFS.

THE DIGESTION OF THE CARBOHYDRATES.

The **Starch** in our diet is converted by cooking into "soluble starch" which is much more readily hydrolyzed by the starch-splitting enzymes or **Amylase**[1] than the uncooked material. The first enzyme to encounter the foodstuffs upon their introduction into the alimentary canal is the amylase or **Ptyalin** of saliva. This enzyme energetically hydrolyzes the starch to maltose, and it is for this reason that starch, when it is held in the mouth, presently begins to taste sweet.

There is no amylase in the **Gastric Juice**, but nevertheless the digestion of starch or glycogen continues for some time in the stomach, because the optimum reaction for amylase is a very faint acidity. The gastric juice itself is strongly acid, in fact, far too acid to permit the action of amylase if this enzyme were received directly into unneutralised and undiluted gastric juice. But various constituents of the diet, and especially the proteins, combine with the **Hydrochloric Acid** of the gastric juice and partially neutralize it, so that the contents of the stomach during the partaking of a meal and the earlier periods of digestion are either neutral or only faintly acid. Long before the acidity of the gastric contents approaches that of pure gastric juice, the pyloric sphincter opens and permits the passage of the semi-digested foodstuffs in small portions at a time into the lumen of the small intestine.

The maltose which is thus formed by the digestion of starch is not normally absorbed from the stomach either as such, or in the form of its further cleavage-product, glucose. Under normal conditions there is little or no absorption of maltose or other sugars from the stomach. If the pylorus be ligated, some absorption of sugar will then be found to occur, but only under conditions involving abnormal dilatation. No carbohydrate-splitting enzymes are found in the gastric juice of man. It is stated that **Lactase** may often be found in the gastric juice of the calf, but not in the adult animal.

After the foodstuffs have remained in the stomach for a sufficiently long period to allow the **Chyme** to become faintly acid through admixture with an excess of gastric juice, the pyloric sphincter opens and permits the passage of the chyme into the upper part of the small

[1] The amylases are frequently referred to as Diastases. In French scientific literature the word "Diastase" is used as a generic term to include all types of enzymes.

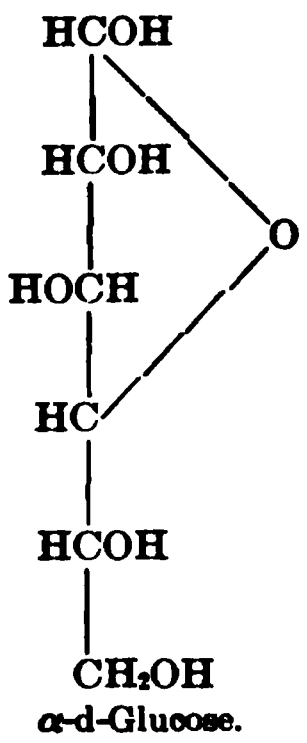
intestine. Here the foodstuffs are very soon met by the alkaline **Pancreatic Juice**, which reaches the intestine, in man, through the common duct of the liver and the pancreas.

The pancreatic juice contains an **Amylase** which completes the work of the salivary amylase and furthermore, a **Maltase** which converts the maltose, derived by the action of amylase from starch, into **Glucose.** The glucose which is thus formed is very rapidly absorbed into the portal circulation, and carried to the liver where it is converted into **Glycogen.** The rapidity of this conversion is very great. Thus the quantity of glucose derived from the polysaccharides in a single meal may very readily exceed one hundred grams. Dissolved in all of the blood in the body, which cannot exceed seven liters in a man of seventy kilos, this would give a glucose concentration of no less than 1.5 per cent. As a matter of fact even at the height of absorption during a meal rich in carbohydrates the concentration of glucose in the blood of a person in normal health never exceeds one-tenth of this. As rapidly, therefore, as the glucose is taken to the liver by the portal circulation, it is transformed into the colloidal anhydride, glycogen, and held in reserve for future consumption.

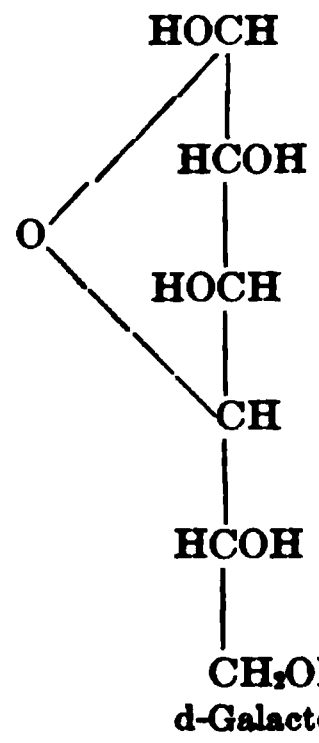
When, however, an extraordinary load is thrown upon this mechanism, by the excessive ingestion of diffusible sugars, some slight degree of **Glucohemia** or excess of sugar in the blood may nevertheless occur and in these cases the glucohemia is relieved by the passage of sugar into the urine. This type of glycosuria is known as **Alimentary Glycosuria.** The sugar which is found in the urine is usually **Glucose,** but when cane-sugar or sweets made of cane-sugar have been ingested in large quantities, **Levulose** may also be found in the urine, together with traces of unhydrolyzed cane-sugar. Lactose is somewhat more readily absorbed and excreted as such than cane-sugar. If either of these sugars be injected intravenously, they appear unaltered and quantitatively in the urine.

It is an exceedingly remarkable fact that whereas amylase and maltase are both present in the digestive juices, **Lactase** and **Invertase** are usually completely absent, or if lactase is present its action is inconspicuous. We have seen that the unaltered disaccharides, if absorbed as such, are not utilized but are as promptly as possible ejected from the circulation by the kidneys. Yet lactose is the sole carbohydrate nutriment of suckling infants and cane-sugar is an exceedingly important item in the dietary of modern peoples. As a matter of fact, although the hydrolysis of these disaccharides cannot be accomplished to any important extent by the secretions which are poured into the alimentary canal by the various digestive glands, yet the consequence of their ingestion is actually the appearance of increased glucose in the portal circulation and enhanced storage of glycogen in the liver. When partaken of in reasonable amounts they are furthermore fully utilized for maintenance and the production of energy in the body. At some point during their passage through the

intestinal epithelium they are evidently broken down into simple sugars or monosaccharides, but the modifications induced by the intestinal epithelium go even further, for the hydrolysis of cane-sugar yields **Levulose** as well as glucose, and the hydrolysis of lactose yields **Galactose** as well as glucose. We are compelled to assume that the levulose and galactose fractions of these molecules are converted, either in the intestinal mucosa or else in the liver epithelium, into glucose. In the case of levulose this presents little theoretical difficulty, for the partial conversion of levulose into glucose can be brought about *in vitro* by the prolonged action of dilute alkali. We are acquainted with no mechanisms, however, which will accomplish the direct transformation of d-galactose into d-glucose, much less with any enzyme which will bring it about. That the converse process, the transformation of glucose into galactose may be brought about in living tissues is shown by the **Glycosuria** which immediately succeeds extirpation of the mammary glands in milch-cows and goats. The sugar that appears in the urine is glucose, and glucose only, although the lactose for the manufacture of which the excess of glucose had previously been utilized, is a compound of glucose and galactose. The remarkable feature of this transformation is that it involves disruption of the oxide-ring of glucose and its reformation upon the opposite side of the molecule:



α-d-Glucose.



d-Galactose.

The normal circulating form of hexose is therefore d-**Glucose** and d-glucose only. Whatever form of hexose or polysaccharide derived from a hexose may be ingested, if it is absorbed at all, it appears under normal circumstances as **Glycogen** in the liver, having either reached the liver-cells in the form of glucose, or else been transformed by them into glucose as a preliminary step in the formation of this colloidal reserve-carbohydrate. From the liver the carbohydrate material is redistributed over the body as the need arises, being broken down to glucose again before it makes its appearance in the circulation. The determining factor which regulates the discharge of this carbohydrate reservoir is probably the concentration of glucose in the blood. This

normally lies between 0.5 and 0.15 per cent. and we may suppose that when the consumption of carbohydrate in distant tissues results in a certain degree of depletion of local stores, and of the circulating glucose, the equilibrium between the glycogen stores in the liver and the glucose in the fluids bathing the liver-cells is disturbed, and glycogen breaks down in order to restore it.

$$\underset{\text{Glycogen}}{(C_6H_{10}O_5)_n} + nH_2O \rightarrow \underset{\text{Glucose}}{nC_2H_{12}O_6)}$$

We must suppose that **Pentoses**, in so far as they form constituents of animal tissues, namely in the d-**Ribose** radical of guanylic and inosinic acids, are derived from pentoses originally contained in the diet. It will be recollected that guanylic and inosinic acids represent fragments derivable by partial hydrolysis from vegetable nucleic acid, and catalytic agents capable of bringing about this cleavage are found widely distributed in animal tissues and tissue fluids The mononucleotids are not improbably absorbed as such, the adenine mononucleotid being transformed by direct deaminization into the corresponding hypoxanthine derivative.

An important proportion of the dietary of herbivora, however, is furnished by **Pentosans**, or polysaccharides derived from pentoses. We have no evidence of the existence, either in the digestive juices or in the epithelial wall of the intestine, of any enzymes capable of transforming these substances into glucose. Yet the experimental fact remains that pentoses can be utilized by animals, and energy derived from them for the performance of work and the maintenance of the body. Whether they are absorbed and oxidized in the tissues as such or as glucose is not known, but the administration of **Rhamnose** to a diabetic whose urine has been made sugar-free by the exclusion of carbohydrates from the diet, leads to reappearance of glucose in the urine.

The **Celluloses** in the dietary are indigestible by any of the enzymes produced by the digestive glands or the intestinal epithelium. Nevertheless they are partially utilized, as much as forty per cent. of young and tender cellulose, such as that occurring in lettuce, being utilizable for the production of energy by human beings. We owe this ability to the digestive activities of the bacteria which inhabit the lower intestine. The bacterial flora is particularly abundant in the lower intestine of the herbivora, and as much as seventy per cent. of cellulose may be dissolved *in vitro* by the intestinal juices of a horse. The products of this digestion are not monosaccharides, but carbon dioxide, methane and fatty acids of which the latter only, of course, are available for nutrient purposes.

The most important function of the celluloses in the diet, however, is that of communicating bulk to the intestinal contents, and promoting peristalsis by affording a favorable consistency and volume for propulsion with a minimum of muscular effort. This function of the

celluloses is most especially important in those animals, such as the herbivora, which have very long intestines. In man, however, too large a proportion of indigestible carbohydrate, as for example in the dietary of vegetarians, may lead to incomplete absorption of the digestible foodstuffs and promote, in this way, bacterial activities to an undesirable extent.

The Digestion of the Fats.—Very slight lipolytic action is exerted by the **Gastric Juice.** Not only is the lipase-content of gastric juice low, or the lipase weak in action, but the fats, while they remain in the stomach, being insoluble in water and in the form of relatively large masses, present only a limited surface of contact with the gastric juice, and the **Lipase** which it contains can hydrolyze the fat-masses only at their surface. However, the slight action which is exerted by the gastric lipase is probably of no little importance, for it ensures that upon the entry of the fats into the upper part of the small intestine they contain a small admixture of fatty acid, which greatly promotes their rapid emulsification by the alkaline fluids with which they here come into contact.

The path of **Absorption** of the fats is quite different from that which is followed by the carbohydrate. Instead of passing into the blood-stream, after having traversed the epithelial lining of the intestine, they are deflected into the lymphatics and carried thence into the thoracic duct. After a meal rich in fats, the numerous small lymphatic vessels coming away from the small intestine are full of milky fluid and stand out distinctly from the surrounding tissues, by reason of their whiteness and opacity, whereas under resting conditions, when digestion is not proceeding, they are transparent and difficult to distinguish.

It is, therefore, possible to follow the absorption of fat from the small intestines by naked-eye examination, and it was in this way that Claude Bernard in 1846, discovered that the **Pancreatic Juice** is essential for the digestion and absorption of fats, for no absorption is evidenced by the appearance of fat in the lacteals until the foodstuffs have reached the point at which the pancreatic duct opens into the duodenum. In man the ducts from the liver and the pancreas join to form a common channel of discharge, in the dog the two ducts enter the intestine very close together, but in the rabbit a considerable interval separates the openings of the two ducts, the **Bile** from the liver being discharged into the intestine at a point considerably above that at which the pancreatic duct opens. In the space between these two ducts no absorption of fat whatever is to be observed after a meal, but immediately below the pancreatic duct the lacteals are seen to be filled with emulsified fat.

Not only the pancreatic juice, but also the bile is essential for the absorption of fat, however, for if by surgical procedures the bile-duct be made to open into the intestine *below*, instead of *above* the pancreatic duct, the lacteals in the space between the ducts, notwithstand-

ing the admixture of pancreatic juice with the foodstuffs, are again seen to be clear and free from fat, while immediately below the new point of entry of the bile-duct into the intestine active absorption of fat is evidenced. Moreover, partial or total occlusion of the bile-ducts through catarrhal conditions or tumors is not uncommon, and this invariably leads to very defective absorption of fat, a large proportion of unabsorbed fat passing into the feces, even when the discharge of pancreatic juice into the intestine has not been interfered with at all.

Two separate factors are therefore essential for the proper absorption of fats, namely the bile and the pancreatic juice. The fat-splitting enzyme, **Lipase**, is contained in the pancreatic juice and not in the bile. The essentiality of the bile in this process arises not from any power of digesting fats which it possesses itself, but from the facilitation of the digestion of fats by pancreatic juice which it brings about.

The fats differ from the other nutritive constituents of the diet in their insolubility in water. The enzyme, lipase, which accomplishes their digestion is, however, not only soluble in water, but secreted and poured into the intestine in a watery medium. To secure contact of these substances of diverse solubilities some special mechanism is required and this is supplied by the **Emulsification** of the fat, partly by the alkaline carbonates contained in the pancreatic juice and the bile, but especially by the **Bile-salts**, sodium glycocholate and taurocholate.

By the action of alkalies and alkaline salts upon partially hydrolyzed fat containing a little fatty acid, **Soaps** are formed, by combination of part of the base in the alkaline salt with the fatty acid.

Na_2CO_3	+	$C_{17}H_{35}COOH$	=	$NaHCO_3$	+	$C_{17}H_{35}COONa$
Sodium carbonate		Stearic acid		Sodium bicarbonate		Sodium stearate.

The presence of a small amount of soap facilitates the formation of an emulsion of fat with water because the soap tends to collect in a film at the surfaces of the oil-droplets and impedes their coalescence into larger drops. The concentration of the soap at the surfaces of the droplets is brought about by the fact that they lower the **Surface-tension** of the oil-water interface, so that, a film having once been formed, if a discontinuity should appear in it, the surface of oil which is exposed will have a greater tension than the surface of soap which covers the remainder of the droplet. The effect of this is to cause the exposed surface, where the film is broken, to contract more forcibly than the remainder and thus pull the edges of the film together again.

The emulsification of the fats in the foodstuffs is thus initiated by the alkaline carbonate in bile and pancreatic juice, forming soaps with the trace of fatty acid arising from lipolytic action of the gastric juice. The emulsifying-power of the soaps is, however, far inferior to that of the bile-salts, which reduce the tension of the oil-water

interface a great deal more than soaps do, and the alkaline salts of the pancreatic juice, unaided by the sodium glycocholate and taurocholate of the bile, are unable to bring about sufficiently rapid and complete emulsification to permit digestion and absorption to occur with the necessary rapidity to ensure total utilization of the fats in a meal. Furthermore the bile-salts, in some way which is not yet fully understood but which also probably arises from reduction of surface-tensions, facilitate the **Absorption** of the fatty acid and glycerol by the intestinal epithelium after the digestion of the fat has been completed.

The emulsification of the fats enormously enhances the area of fat and, therefore, the number of fat-molecules which are exposed simultaneously to the action of lipase. Thus one cubic centimeter of oil floating upon the top of water in a test-tube which is one centimeter in diameter will be in contact with the water over an area of 0.785 square centimeters. The same volume of oil, broken up into ten thousand droplets and distributed through the water would expose a surface of no less than a hundred square centimeters to contact with substances dissolved in the water. Hence the droplets formed by emulsification are rapidly eroded and finally consumed and converted into fatty acids and glycerol by the **Lipase** in the pancreatic juice.

The carbohydrate and the proteins are broken up by the digestive ferments and the intestinal epithelium into their simple constituents, and these are absorbed and carried to the liver as such, to be subsequently distributed therefrom over the body. The fats, on the contrary, reach the blood through the lymphatic circulation without preliminary elaboration or reassortment by the liver. Corresponding to this fact we find that they are thrown into the circulation, not in the form of their simple components, but in the comparatively elaborate form of **Neutral Fat.** The fatty acid and alcoholic radicals of the original fat are, in fact, quantitatively recombined within the brief period of their passage through the substance of the intestinal epithelium, and the work of digestion is completely undone again before the fat appears in the lacteals.

It was inevitable that the appreciation of this fact by investigators should suggest the question whether and to what extent the preliminary hydrolysis of fats by lipase is essential. If the hydrolysis of fat is completely reversed within so brief a period and distance, is the hydrolysis itself a necessary prerequisite to absorption?

Much investigation has been devoted to this problem, and as a result we are in possession of a variety of results arising out of different methods of attack. These results indicate that notwithstanding the fact that the hydrolytic splitting of the fats is so temporary it is nevertheless essential. Thus fats which are not hydrolyzed by lipase, and cholesterol esters, waxes and hydrocarbon oils which simulate fats in their solubilities and other physical characteristics, but are not decomposed by lipase, are not absorbed to any measurable extent. Even when vaseline or liquid petrolatum are administered in emulsions,

formed by mixing them with small quantities of an emulsifiable fat or oil, over ninety-five per cent. of the hydrocarbon is recoverable, unaltered, in the feces. The same is true of **Lanoline** which consists of a mixture of cholesterol esters of fatty acids and is not saponifiable by lipase. These esters are not absorbed; they pass into the feces unaltered, although free cholesterol itself can be shown to undergo absorption.

Fatty acids which are normally foreign to animal tissues are absorbed, but only when the fat is previously split into its constituents by lipase. This has been most conclusively shown by the following very beautiful experiment of Bloor's. Bloor prepared the fatty-acid compounds of the polyatomic alcohols derivable from sugars by reduction. Among these **Dilaurate of Isomannitol** has a high dextrorotation, while the normal body-fats are optically inactive. On administering this substance to dogs in their food and collecting the chyle from the thoracic duct a large proportion of fat was obtained which yielded **Lauric Acid** on hydrolysis. Lauric acid is not found normally in animal fats, and it must therefore have been derived from the isomannitol dilaurate which had been administered. But the fat obtained from the thoracic duct was also **Optically Inactive**; therefore, it cannot have consisted of isomannitol dilaurate. The accuracy of the method was sufficient to have detected the absorption of 0.5 per cent. of the isomannitol dilaurate which had been administered. Not more than this proportion therefore can have been absorbed without previous hydrolysis by the pancreatic lipase.

The carbohydrates, and, as we shall see, the proteins are absorbed in the form of the simplest components into which they can be converted by the hydrolyzing enzymes, and are distributed to the tissues after having passed through the liver. The fats, on the contrary, are thrown directly into the circulation in a comparatively complex form. Corresponding to this difference in the method of distribution we find that the composition of the tissue-fats is very much more dependent upon the varying nature of the diet than the tissue-carbohydrates or the tissue-proteins. Thus **Erucic Acid**, $C_{21}H_{41}COOH$, is never normally present in the tissue-fats of dogs. Yet if rape-seed oil, which contains notable quantities of erucic acid glyceride, be given to starving dogs, this fatty acid may subsequently be isolated from their tissues. The normal melting-point of dog-fat is 20° C. Munk allowed a dog to fast for nineteen days, until the tissues were presumably free of reserve-stores of fat. The dog then weighed sixteen kilograms. It was now fed for fourteen days with mutton tallow. The weight of the animal increased during this period by seventeen per cent. On "trying out" the tissues 1100 grams of fat were obtained and its melting-point was 40° C. The administration of the fat of high melting-point had, under these conditions, led to an abnormally high melting-point of the fats laid up in the tissues.

The results which we have quoted were obtained with starving animals. Under normal conditions, however, when the tissues are not

depleted of fat-reserves, they are able to exert some measure of control over the nature of the fats which they assimilate. Munk investigated a patient who was afflicted with a fistula from which it was possible to collect the chyle before it entered the blood-stream. When this patient was fed upon a diet containing no other fat than mutton-fat, the chyle-fat had nevertheless a lower melting-point than mutton-fat. When the patient was fed with no fat at all but **Cetyl Palmitate**, which melts at 55°, the chyle-fat was found to melt at 36° and to consist of a mixture of **Glyceryl Tripalmitate** and glycerides of **Oleic Acid** to the extent of fourteen per cent. of the mixture, adjudged by the absorption of iodine by the mixed fats. Hence under normal conditions a measure of control is exerted by the intestinal wall itself and a proportion of glycerol and oleic acid may be furnished to supplement the deficiencies of the dietary. This of course becomes impossible if the glycerides of oleic acid which are present in normal tissues have been previously depleted by starvation.

The fats may also be modified in the opposite direction and the proportion of oleic acid glycerides reduced during their transmission through the intestinal epithelium. Thus when **Cod-liver Oil**, which contains a great excess of unsaturated fatty acids, is administered to dogs, the iodine number of the fats after absorption is less than that of the fat in the food.

The absorption of fat leads to a temporary increase in the fat content of the blood, where it is held in a finely emulsified condition. The ingestion of fat-rich foods, as for example, cream, may result in an increase of the fat-content of the blood to no less than six times the normal concentration during the intervals between absorption. Under such circumstances the blood-serum obtained by centrifugalizing defibrinated blood is often cloudy with suspended fat and globules of fat may not infrequently be found floating upon the top of the column of fluid in the centrifuge-tube. Ultimately the excess of fat disappears from the blood, the neutral fats having been built up into the fatty connective tissues. The greater part of the fat-reserve is contained in special fat-cells, in which the fat appears, at first in small globules, and later in larger globules which coalesce until the accumulation of fat forces the protoplasm into the periphery of the cell, so that it presents an annular appearance on cross-section, with the flattened nucleus forming a slight thickening of the ring at one side. Occasionally such cells disintegrate bodily, it being in this way that the solid constituents of **Milk** are formed in the mammary glands.

Upon allowing fat-rich blood to stand in laboratory-glassware at body-temperatures a proportion of the fat becomes diffusible. The nature of the change which occurs is not yet understood, nor is it certain whether or not this, or a similar change in the properties of circulating fat precedes its absorption by the tissues. Under certain pathological conditions, and particularly in **Diabetes**, the percentage of fat in the

blood is greatly increased above the normal, a condition which is known as **Lipemia.** It is stated that in these cases the power of the blood to render fats diffusible is diminished, the excess of fat being present wholly in the emulsified, or non-diffusible condition.

The **Lecithins** are very readily and rapidly split by lipase into fatty acids and **Glycerophosphoric Acid.** This latter compound, however, is not split by the digestive juices. It is not known whether hydrolysis necessarily precedes the absorption of the lecithins. The rapidity with which they are split by lipase indicates that at any rate a large proportion of the lecithins must inevitably be hydrolyzed before absorption can be completed. On the other hand the extreme solubility of lecithins in solutions of bile-salts encourages the view that a part at least of these substances may be absorbed without preliminary digestion, and this view is further supported by the remarkable effects of egg-lecithin upon the growth and development of animals and upon the nitrogenous balance, when it is administered by mouth, effects which, as yet, have not proved possible to evoke by the administration of the constituent parts into which it disintegrates upon hydrolysis. It is, however, possible that these effects of administering lecithin may be attributable, not to lecithin itself, but to impurities which are commonly associated with crude preparations of lecithin.

Cholesterol has been definitely shown to be absorbed as such. If an abnormal quantity of cholesterol be administered by mouth to animals, the excess is laid up in certain tissues, particularly those of the liver, spleen and suprarenal gland, and serious lesions may result from the accumulation of these deposits. Certain organs, *e. g.*, the kidneys, remain free from cholesterol deposits even when cholesterol is administered in very great excess, but if lesions arise from some other cause, for example if **Nephritis** is induced by the administration of uranium salts, then cholesterol tends to become deposited in the injured tissues. In rabbits, but, so far as has yet been ascertained, not in other species of animals, the administration of excess of cholesterol is followed by the formation of large deposits in the intima of the arterial walls, particularly in the wall of the aorta, leading to the formation of atheromatous lesions resembling those which are observed in cases of **Arteriosclerosis.**

The **Cholesterol Esters,** however, are not saponifiable by lipase and are not absorbed. Hence **Lanoline,** administered by mouth, is recoverable quantitatively in the feces.

The **Bile-salts,** which serve as a vehicle and adjunct in the absorption of the fats, undergo a partial circulation in the body, for after entering the small intestine through the channel of the bile, they are partially reabsorbed during their passage with the foodstuffs down the intestine. Thus **Glycocholic Acid** is nearly absent from the bile of carnivora, but on administering this bile-acid to carnivorous animals, it appears in important quantities in their bile.

THE DIGESTION OF THE PROTEINS.

Until a very few years ago it was generally held that the proteins were absorbed from the stomach and intestine in the form of **Peptones**, and, indeed, prior to the first years of the present century, it was believed in many quarters that not merely peptones, but even unaltered protein or at least infraproteins resulting from the very earliest cleavage of the native protein molecule might be absorbed without further hydrolysis.

This view of protein-absorption stood in rather striking contrast to our knowledge of the absorption of other foodstuffs which were at a comparatively early date known to undergo complete or nearly complete hydrolysis prior to their absorption. Moreover, the elaborate machinery of enzymes for the splitting of proteins not only to peptones, but to amino-acids which is provided by the digestive organs would appear, if the older view were correct, to exist without any necessary purpose or function. Considering these facts it may appear strange that so exceptional a view of protein-absorption should ever have gained general acceptance; but, as usual in the historical development of science, a misinterpreted experiment furnished the foundation for an extensive edifice of erroneous hypothesis.

The observation which led us astray was the outcome of an experiment by Voit, who, in 1869, showed that undigested proteins, unmixed with gastric or pancreatic juice, rapidly disappear when they are introduced into a ligated loop of small intestine, while a little later it was further found by Hofmeister that proteoses and peptones similarly disappear when introduced into an isolated loop of intestine. The latter of these observations received its correct interpretation when Cohnheim, in 1901, showed that the **Succus Entericus** which is secreted by the mucous membrane of the small intestine, contains an enzyme, **Erepsin**, which hydrolyzes proteoses and peptones to amino-acids, leaving, however, native proteins with the exceptions of casein and the protamines, unattacked. The disappearance of peptone from an intestinal loop is therefore accounted for by its hydrolysis by erepsin into amino-acids. The disappearance of native proteins such as egg-albumin from isolated loops of intestine is, however, a more difficult matter to interpret, and it cannot yet be said to have been completely elucidated. It is, however, certain that under normal conditions unaltered proteins never reach the circulation by absorption from the intestine for the following reasons:

In the first place, when native proteins are injected into the circulation, a proportion of the protein thus introduced appears in the urine. Evidently it is treated as a foreign constituent of the blood and discharged, in so far as that is possible, by the kidneys. Another portion of the protein is discharged by the kidneys in a non-coagulable form which is still, nevertheless, a protein. At the same time there is a marked increase of proteose-like substances in the blood and some increase of the urea-output.

To some extent, but a very limited extent therefore, parenterally introduced protein, that is, protein injected directly into the circulation, may be utilized by the tissues, since a proportion of the protein is evidently converted into a normal product of protein catabolism, namely, urea. But it is also evident that the utilization of protein thus introduced is imperfect, that it is abnormal because the urine contains protein which is not the case when proteins are absorbed from the digestive tract, and that the utilization of the protein which does occur is preceded by hydrolytic cleavage. Moreover it is not even certain that the additional urea-output which results from the injection of foreign proteins is due to utilization of the protein itself, since it has been found by Mendel and Rockwood that the introduction of a foreign protein, such as **Edestin** or **Casein** into the circulation leads to a considerably more than proportionate increase of the nitrogenous secretion, in other words to actual destruction of tissue-proteins.

In the second place, the intravenous or subcutaneous injection of proteins which are foreign to the tissues of the animal receiving them, results in the production of a variety of specific **Antibodies** or substances appearing in the circulation which have the property of precipitating or otherwise modifying the protein employed for injection. If the injections are repeated, and successive injections are separated by only a few days from one another, the result after some weeks is the production of a specific **Precipitin** which circulates in the blood of the immunized animal, so that if the blood-serum of the animal be now mixed with a solution of the protein which was employed for injection, that protein, but no other, is precipitated. If a single injection be made and then a second only after a considerable interval, *e. g.*, three or four weeks, the effect of the second injection is to induce **Anaphylactic Shock**, a condition which so strikingly resembles peptone-poisoning that many investigators are of the opinion that it is due to the development in the tissues of the sensitized animal of an enzyme having the specific ability to rapidly break down the particular protein employed and to convert it into proteoses or peptones.

Now it has been shown that even after the introduction of excessive amounts of native protein into an isolated loop of intestine, although the protein disappears and would seem to have been absorbed, yet no evidence is obtainable of the development of antibodies in the circulation of the animal so treated, nor is there any sensitisation, so that a second dose of the protein, after a considerable interval, does not give rise to symptoms of anaphylactic shock.

There can be little doubt therefore that proteins are not absorbed without previous hydrolysis, and there is much ground for supposing that even that proportion of parenterally introduced protein which is utilized by the tissues, is utilized simply because it has been excreted into the intestine and reabsorbed therefrom after digestion.

The case against the direct absorption of peptones from the intestine

is an even stronger one, for a considerable proportion of the proteoses and peptones arising from the incomplete hydrolysis of proteins are definitely toxic, and the absorption of this type of protein digestion-product would lead to incessant food-intoxication. It is true that peptones may be absorbed from a *ligated* stomach, and peptones and proteoses resulting from secondary degenerative changes in the intestinal epithelium may be absorbed after prolonged ligation of an intestinal loop, and may produce severe symptoms of peptone intoxication. These, however, are circumstances not in the least comparable with those attending normal digestion, for a ligated and distended stomach becomes permeable, as we have seen, to carbohydrates which are normally not absorbed until they reach the intestine, so that the permeability of the gastric mucosa is evidently deranged by this process. The absorption of toxic proteoses from an intestinal loop is attained only after very prolonged ligation, and while this condition may be comparable with that prevailing in severe intestinal stasis, it is certainly not comparable with the normal phenomena of digestion and absorption.

In certain cases, which must be admitted to be exceptional, individuals may display phenomena of **Anaphylaxis** after the ingestion of particular proteins toward which the individual has an idiosyncrasy. Thus, hypersensitiveness to the proteins of horse-serum is not unusual and is responsible for the majority of cases of **Serum-sickness** and deaths arising from the use of **Diphtheria Antitoxin** prepared from horse-serum. It is especially frequently displayed by chronic asthmatics. A smaller proportion of individuals are hypersensitive to the proteins in the white of egg and betray symptoms of anaphylactic shock, such as **Asthma**, when they have partaken of eggs. Others, again, are hypersensitive to the protein in strawberries, others to the proteins in shell-fish and so forth. In all of these cases we must assume, from the character of the symptoms, that the absorption of a proportion of unaltered, native protein is responsible for the disorder. The proportion of protein absorbed which would suffice to account for these effects, however, is excessively small. Thus Wells has shown that the injection of such a minute amount as one millionth of a gram of crystallized egg-albumin will sensitize a guinea-pig, so that a subsequent intravenous injection of no more than one-tenth of a milligram of the same substance will lead to fatal shock. The comparative rarity of these phenomena of protein-intoxication, and the minute proportion of absorption of unaltered protein which would evidently suffice to evoke them, afford eloquent testimony to the difficulty with which native proteins and peptones are absorbed without preliminary digestion.

We are thus brought indirectly and by the exclusion of other possibilities, to the conclusion that the proteins of the diet must be completely broken down into **Amino-acids** prior to their absorption. Direct evidence of the correctness of this view has, however, been obtained in recent years in a variety of ways, of which the following are the more important.

In the first place it has been ascertained, thanks to the researches of London, that during the normal digestion of proteins in the intestine large proportions of amino-acids are actually formed, and progressively absorbed subsequently to their formation. The experiment consisted in establishing a number of fistulæ at intervals along the intestinal tract, so that samples of the intestinal contents could be withdrawn and examined after varying periods of intestinal digestion. The animal was fed with measured amounts of pure proteins and the digestion-products obtained from the successive sectors of the intestine (Fig. 7). It was found that these samples contained notable amounts of amino-acids and, moreover, that the relative proportions of the amino-acids arising from the dietary protein differed in different sec-

FIG. 7.—Dog with intestinal and glandular fistulæ. (After London.)

tions of the intestine. Thus when the animal received the protein **Gliadin**, the duodenum contained 0.75 grams of tyrosine to 2.5 grams of glutamic acid; the jejunum contained only 1.1 grams of tyrosine per 20.9 grams of glutamic acid and the ileum contained only a trace of tyrosine as contrasted with 33 grams of glutamic acid. Evidently therefore, not only are amino-acids formed in the normal intestinal digestion of proteins, but they are *absorbed selectively, e. g.*, in the particular case in point, tyrosine was absorbed from the intestine much more rapidly than glutamic acid.

In the second place it has been shown by Folin and Denis, that if amino-acids are introduced into a loop of intestine the non-protein nitrogen of the blood is decidedly increased during the period that absorption might be supposed to be occurring, and the origin of this

increase was subsequently demonstrated by Abderhalden, who, by employing an enormous volume, fifty liters of blood, succeeded in demonstrating the presence therein of the amino-acids glycine, alanine, leucine, valine, proline, aspartic acid, glutamic acid, arginine, histidine and lysine.

It has furthermore been shown by Abderhalden, Henriques and others that animals may be maintained in perfect nitrogenous equilibrium for prolonged periods on a diet containing no other source of nitrogen than amino-acids. It is necessary, however, to include in this diet *all* of the amino acids-which contribute to the composition of the various tissue-proteins. The omission of **Tryptophane**, for example, leads to daily loss of weight and, in effect, to nitrogen-starvation. Nitrogen equilibrium and even nitrogen retention, *i. e.*, accretion of tissue, was secured by Abderhalden in a dog to which a diet was administered containing the following admixture of amino-acids as the sole source of nitrogen: Glycocoll 5 grams, d-alanine 10 grams, l-serine 3 grams, l-cystine 2 grams, d-valine 5 grams, l-leucine 10 grams, d-isoleucine 5 grams, l-aspartic acid 5 grams, d-glutamic acid 15 grams, l-phenylalanine 5 grams, l-tryosine 5 grams, l-lysine 5 grams, d-arginine 5 grams, l-proline 10 grams, l-histidine 5 grams, l-tryptophane 5 grams. The daily ration of amino acids therefore weighed 100 grams and contained 13.87 grams of nitrogen. It approximately resembled in composition the mixture of products which results from the hydrolysis of the proteins of muscular tissue.

We have seen therefore: (1) That amino-acids are formed in important proportion in the intestinal digestion of proteins. (2) That amino-acids may be absorbed from the intestine and, (3) that amino-acids suffice to supply the nitrogenous needs of the body. We may infer that the absorption of amino-acids is a normal and probably the only normal method whereby the materials for the synthesis of proteins are conveyed to the tissues.

The difficulty of demonstrating the presence of amino-acids in the blood after the absorption of the digestion-products of protein arises from two sources: firstly the slowness of absorption and the rapidity of circulation, which results in extreme dilution of the amino-acid products which enter the portal venous system, and secondly the rapidity with which the amino-acids in the blood are absorbed from it by the tissues. The amino-acids are therefore present in the blood even during the height of absorption only in very small concentrations and, to add to the difficulty of the problem, the blood is a fluid which is very rich in nitrogenous substances, proteins, which interfere to a serious extent with the chemical manipulations which were formerly necessary for the determination of small concentrations of amino-acids. In recent years the development of our technical knowledge has simplified and enhanced the accuracy of our methods of estimation and, in particular, the development of the nitrous-acid method of estimating amino-nitrogen immediately enabled us to detect with

ease and certainty the accumulation of amino-acids in the blood which accompanies absorption after a meal rich in protein.

The absorption of amino-acids from the intestine and their consequent presence in the blood has also been very beautifully demonstrated by Abel, employing his method of **Vividiffusion.** This method consists in deflecting a fraction of the blood-stream and causing it to pass through a series of collodion-tubes before returning to the general circulation. The collodion-tubes are immersed in a salt solution of the same concentration as the inorganic salts in the blood, so that diffusible substances other than inorganic salts dialyze out of the blood into the saline solution. By renewal of the saline solution considerable quantities of the diffusible substances in blood may be collected and, among others, various amino-acids (Fig. 8).

Fig. 8.—Abel's vividiffusion apparatus. (After Macleod.)

It is thus evident that the protein constituents of the dietary are absorbed into the circulation in the form of their amino-acid components, some selection of the amino-acids transmitted to the blood being exercised by the intestinal epithelium. The question now arises, where, and in what way, these amino-acid fragments are resynthesized into protein.

Amino-nitrogen determinations show that the excess of amino-acids which accumulates in the blood after a meal very rapidly disappears, while coincidently a considerable increase in the free amino-acids is found to have taken place in the tissues. The amino-acids are there-

fore stored up in the tissues. There is a limit, however, to the capacity of the tissues to retain amino-acids and this upper limit of capacity varies with different tissues. Thus the upper limit in the case of muscular tissues is about 75 milligrams of amino-nitrogen per hundred grams of tissue. This characteristic limit cannot be overstepped and if the quantity of amino-nitrogen brought to the tissues exceeds their **Assimilation-limit** the excess of amino-acids in the blood is destroyed by **Deaminization**, the nitrogen being split off in the form of ammonia which is converted by the liver into **Urea**. The **Liver** plays a leading part in this process and continually and rapidly desaturates itself by deaminization of the free amino-acids which it contains; doubtless other tissues share this ability, but the power of the liver to deaminize amino-acids is certainly in excess of that of other tissues, because, although other tissues do not show any greater avidity than the liver for amino-acids, and do not reach a higher saturation-limit of amino-nitrogen, yet within a few hours after the saturation of all the tissues with amino-acids which succeeds a protein-rich meal, or injection of amino-acid mixture, the other organs all contain more amino-acid material than the liver. When we consider, also, that the liver is an exceedingly bulky organ, its possession of a high deaminizing power ensures its overwhelming predominance in this process.

When, for any reason such as that afforded by degenerative changes, the deaminizing power of the liver is impaired, this mechanism for disposing of undue excess of amino-acids may prove insufficient and the kidneys may assist by excreting unaltered amino-acids. If, at the same time, the introduction of amino-acids into the blood is unnaturally rapid, as for instance by rapid injection of large amounts of amino-acids in solution, the mechanisms for their disposal may prove to be entirely inadequate and serious symptoms of intoxication, or even death may ensue.

The absorption of amino-acids from the blood is never complete, and free amino-acids are still present in the blood even when the amino-acid concentration in the tissues is far below the saturation-limit. Evidently, therefore, we are dealing here with an equilibrium, somewhat resembling the partition of a dissolved substance between two immiscible solvents, increase in the amino-acid content of the blood leading to increase, up to the saturation-limit, of the amino-acid content of the tissues, while the loss of tissue-protein which occurs in **Starvation** indicates that diminution of the amino-acid content of the blood may also lead to desaturation of the tissues by the passage of amino-acids into the blood. The same mechanism, also, permits transfer of particular amino-acids from one tissue to another and explains the otherwise surprising fact that certain tissues, for example malignant growths, may grow at the expense of other tissues, and also, in part, the fact that the loss of weight of the various organs and tissues in starvation is very unequal, certain tissues losing very heavily while others retain their weight very nearly undiminished until death ter-

minates the process. It will be recollected that a similar equilibrium between the tissues and the blood obtains in the case of glucose and its anhydride, glycogen, a subnormal glucose concentration in the blood leading to the splitting up of glycogen and liberation of glucose by the liver to replenish the blood and thereby the muscular tissues to which it carries the carbohydrate fuel which furnishes the energy-equivalent of muscular work. In the same way we may suppose that the amino-acids in the tissues stand in a relation of equilibrium to the amino-acids in the blood, on the one hand, and to the proteins of the tissue on the other.

The proteins which are found in the various tissues of the body are highly specialized and characteristic of the tissue-elements in which they occur. The proteins in the various **Connective Tissues** are especially diverse in their composition and characteristics. Thus the proteins of fibrous tissue are extraordinarily rich in glycocoll, and those of elastic tissue are especially rich in glycocoll and also in glutamic acid. Among other highly specialized proteins may be mentioned the keratin of horny epidermal tissues which is exceptionally rich in cystine, the protamines which are exceptionally rich in diamino-acids, and the mucins which contain an amino-carbohydrate radical. In a less degree the proteins of every type of tissue and cell betray, either in biological or physical behavior or directly, in chemical composition, evidence of distinctive architecture.

The question of the locality of **Protein Synthesis** has evoked a very great deal of discussion and prompted a variety of investigations. Arguing that only the normal blood-proteins, the serum-albumins and serum-globulins could be tolerated in the circulation, and assuming that the amino-acids were not, as we now know that they are, absorbed as such, Abderhalden supposed that the amino-acids which result from digestion are synthesized into protein in the intestinal epithelium, just as the fatty acids and glycerol are synthesized into fats during their passage through the intestinal wall, but with this difference, namely, that whereas the fats which are thus synthesized bear a very close relationship to the fats which were present in the diet, the protein which was presumed to be synthesized must be limited to the blood-proteins characteristic of the species.

This hypothesis, however, made it necessary to view the process of protein synthesis as a very roundabout and uneconomical one, for since the proteins of the tissues differ so markedly from one another and also from the blood-proteins, the blood-proteins evidently could not be built up directly into tissue-proteins, but must first be broken down in the tissues themselves, their amino-acids resorted and rearranged, and resynthesized into the characteristic proteins of the tissue in question. Thus the synthetic work of the intestine would have to be undone again in each of the tissues. Moreover in many of the tissues the process of redegradation and resynthesis would involve an extraordinary amount of waste of amino-acid material. For example,

the proteins of connective tissues could not be synthesized at all from serum-albumin, because it contains no glycocoll, and even their synthesis, from serum-globulin would involve a great deal of wastage, because, whereas serum-globulin contains only 3.5 per cent. of glycocoll, the connective-tissue proteins contain about twenty per cent., so that not less than six molecules of serum-globulin would have to be destroyed to build up one molecule of connective-tissue protein, and the greater part of the remaining amino-acids in these six molecules would not be needed. What, then, is to become of them? They might be locally deaminized, but the predominant deaminizing tissue is that of the liver. In order to reach the liver the rejected amino-acids would generally have to travel thereto in the circulation. If, on the contrary, the amino-acids rejected by the connective tissues are utilized by another tissue, then in order to travel from the one tissue to another they must again enter into the circulation. Whichever hypothesis we adopt we are therefore compelled to revert to the presence of amino-acids in the blood.

The discovery that the amino-acid products of digestion are actually absorbed into the blood-stream as such, and are absorbed from the blood by the tissues, removes these complexities from our interpretation of the process of protein synthesis. It seems most reasonable to suppose that each tissue synthesizes its own individual proteins, and that it is able to utilize for this purpose all of the amino-acids which it absorbs for the reason that the characteristic composition of each individual tissue-protein is already determined by the characteristic admixture and proportion of the various amino-acids which that tissue absorbs and holds in equilibrium with the blood, on the one hand, and with the tissu -protein itself on the other. The individual characteristics of the proteins of the various tissues are therefore determined, in ultimate analysis, by the relative **Permeability** of the tissue in question for various amino-acids, *i. e.*, by the relative ease with which the amino-acids traverse the boundaries which demarcate the tissue.

It is not unlikely that this mechanism of two-sided equilibrium is limited in its powers and that it is for this reason that it is safeguarded or assisted by a degree of preliminary selection by the **Intestinal Epithelium.** It will be recollected that the experiments of London show that if a protein differing very widely from animal tissue-protein be administered, certain amino-acids are absorbed selectively. Thus from **Gliadin,** tyrosine is absorbed much more rapidly than the glutamic acid which it contains in notable excess. The composition and general nutritional standard of the tissues is therefore determined by the following interrelated factors which are severally in equilibrium: (1) The selective absorptive activities of the intestinal epithelium. (2) The general average concentration of food-products in the blood, *i. e.*, the abundance of the dietary. (3) The deaminizing activity of

the various tissues and particularly of the liver. (4) The "saturation-limit" of the tissues for amino-acids. (5) The relative velocities of the opposed processes of protein synthesis and degradation in the several tissues of the body. Of these factors, two main groups may be recognized. Absorption and deaminization on the one hand, determining the abundance of nutrient material in the circulating medium, and the excess or defect of the velocity of synthesis in comparison with that of degradation in the tissues, on the other, determining the rapidity with which the available nutrients are utilized. The former factors are largely subject to environmental influence, for example that of the abundance of the dietary. The latter factors are individually characteristic of the organism, and in turn of the several tissues of which the organism is composed. Two main groups of factors, therefore, contribute to determine the nutrition, composition and growth of organisms, an *Environmental Group* and an *Internal Regulatory Group*. We shall see when we come to the consideration of the problem of Growth (Chapter XX), that the diverse significance of these two groups of factors may very clearly be recognized in the processes of development.

We have seen that the intestinal digestion of proteins leads to the production of amino-acids, and that these are absorbed into the blood-stream as such. A considerable degree of preliminary digestion of proteins is, however, achieved by the **Pepsin** in the gastric juice, and the question therefore arises as to whether any digestion-products of proteins are absorbed from the stomach?

This question may be answered in the negative. We have already seen that under normal conditions neither carbohydrates nor fats are absorbed from the stomach and, analogously, protein digestion-products are not absorbed from the stomach. It is true that carbohydrates may be absorbed from a ligated stomach and so, also, may proteoses and peptones, but this constitutes a condition which is nowise analogous to the conditions which pertain in normal digestion. The non-absorption of protein digestion-products from the stomach is in the first place guaranteed by the fact that the products of protein hydrolysis by pepsin are proteoses and peptones, not amino-acids. It would not be altogether safe, of course, to argue from the inability of pepsin to digest peptones *in vitro* to a similar inability upon the part of the stomach *in situ*, but the studies of London have shown that the production of proteoses and peptones is, in actuality, the main result of gastric digestion. This investigator has established in animals a fistula opening into the intestine immediately below the pyloric sphincter of the stomach. From this fistula it is possible to collect samples of the stomach contents the moment after the completion of gastric digestion, and their ejection into the intestine. The samples not only failed to contain any amino-acids, but the larger proportion of the nitrogen was present in the form of **Proteoses**, and only a lesser propor-

tion in the form of the further cleavage-products, the **Peptones.** The following are typical results obtained:

Protein in the diet.	Percentage of proteoses on completion of gastric digestion.
Egg-albumin	72.5
Gliadin	67.7
Edestin	60.3
Casein	59.1
Gelatin	50.6
Serum-albumin	46.1

the remainder of the protein having reached the peptone-stage of cleavage. With varying quantities of the same protein a definite proportion of proteoses is always formed, as the following results illustrate:

Quantity of gliadin in a meal. Grams.	Percentage of proteoses on completion of gastric digestion.
25	80.8
50	86.1
75	86.5
100	84.9

The significance of gastric digestion lies in the preparatory work which it accomplishes for the intestinal and pancreatic enzymes. The hydrolysis of proteins by **Trypsin** is much more rapid and complete if the protein has been subjected to preliminary digestion by pepsin, and the hydrolysis of proteins to the peptone and proteose stage, furthermore, converts the protein foodstuffs into forms open to attack by the **Erepsin** in the succus entericus. The superior velocity and thoroughness of intestinal protein digestion to the digestion of protein *in vitro* by pancreatic trypsin is attributable in large measure to the fact that the various proteolytic enzymes act in conjunction or succession upon the protein foodstuffs in the alimentary canal, and also to the fact that the *products* of digestion are removed almost as rapidly as they are formed.

In addition to the conversion of proteins into proteoses and peptones, the gastric juice has the special property of converting the casein of milk into **Paracasein.**[1] Paracasein has recently been shown to be derived from casein by partial hydrolytic cleavage, the paracasein molecule representing one-half the casein molecule. Paracasein resembles casein very closely in its general properties and behavior, but its calcium salt is rendered insoluble by a very slight excess of calcium ions at a much lower temperature than the corresponding salt of casein itself. If a sufficiency of calcium chloride, for example, be added to a solution of calcium caseinate, the protein salt will be

[1] In British scientific literature these substances are termed, respectively, Caseinogen and Casein. The word casein, therefore, means the unmodified protein of milk, in American literature, and the infraprotein derived therefrom by the action of Rennin, in British literature. The American nomenclature is to be preferred because it possesses the claim of priority, and is that generally employed in other languages.

precipitated at ordinary temperatures. If a little less calcium chloride be employed, it will remain in solution at ordinary temperatures, but will form a curd on elevating the temperature. Even in the absence of free calcium ions a solution of calcium caseinate becomes markedly opalescent on heating to 45° C. The calcium salt of paracasein is, however, for a like concentration of free calcium ions, clotted or curdled at a lower temperature than calcium caseinate. The presence of free calcium ions is therefore necessary to permit the clotting of milk by gastric juice or extracts of the gastric mucosa. They are not necessary, however, for the conversion of the casein into paracasein, which occurs just as readily in a medium free from calcium ions as in one which contains them; but visible evidence of the change which has occurred is lacking until calcium salts are added. Thus if excess of ammonium oxalate be added to milk, the free calcium ions are removed, through the formation of calcium oxalate. The calcium combined with the casein is unaffected because it is not ionized. On adding **Rennet** (extract of gastric mucosa) or gastric juice in small amount and warming the mixture to body temperature no visible change in the milk occurs. The mixture may be heated to boiling to destroy the enzyme without causing any precipitation or clotting of the milk, but on adding soluble calcium salts, after cooling, clotting of the milk instantly occurs. The calcium is necessary, therefore, merely to render the product of the enzyme action insoluble; not to enable the enzyme to act upon the casein. The part played by calcium in this process is therefore sharply in contrast to the part which it plays in the coagulation of the blood.

It has long been supposed that this change in the properties of casein which is brought about by gastric juice is due to a special enzyme, which is termed **Rennin** or **Chymosin.** Evidence has accumulated in recent years, however, tending to show that rennin is, in fact, identical with **Pepsin** and that rennet preparations which are devoid of power to digest proteins other than casein represent merely pepsin, weakened so greatly as to have lost ability to hydrolyze the majority of proteins at any appreciable speed. Thus, although pepsin and rennin are found in a great variety of situations both in the animal and in the vegetable kingdoms, yet they are invariably found to be associated with one another in the same tissue or tissue-fluid. The close relationship of rennin action to pepsin action is also shown by the following experiment of Morgenroth's. If mixtures of calcium caseinate containing free calcium ions and rennet are kept at low temperatures no coagulation occurs, but slow digestion of the casein (proteose production) does occur. If, however, these mixtures be heated to 20° C. they clot immediately. Thus the process which underlies the clotting, has taken place during the digestion which occurs at low temperatures, but it cannot be visibly evidenced by clotting until the temperature is raised.

THE TIME- AND MASS-RELATIONS OF DIGESTION AND ABSORPTION.

The **Intestine** is an extraordinarily efficient organ of absorption. As much as seventy or eighty per cent. of the total length of the jejunum and ileum may be removed and, provided fats be not too abundant in the diet, absorption still remains practically complete. If the food contains a large proportion of fat, however, over twenty-five per cent. of the fat may, under these conditions, be discharged unabsorbed in the feces as contrasted with four or five per cent. in normal animals of similar kind and dimensions.

The greater part of absorption takes place in the upper part of the small intestine and absorption is practically complete before the contents of the small intestine are discharged into the cecum. There are, however, certain exceptions to this rule, mainly furnished by difficultly digestible foodstuffs. Thus uncooked white of egg is digested with great difficulty and as much as seventy per cent. of this protein may pass undigested and unabsorbed into the large intestine where it may be presumed to afford a favorable culture medium for putrefactive bacteria. Then, also, when proteins very diverse in composition from the normal tissue-proteins of animals, such as certain vegetable proteins, are partaken of, the selective absorption which occurs may result in a proportion of an amino-acid which is present in undue excess remaining unabsorbed until its passage into the large intestine.

The **Stomach**, as might be imagined from the nature of the part it plays in digestion, is not essential to the absorption of foodstuffs. Excision of the stomach is followed by a good utilization even of proteins, the digestion being accomplished by the trypsin of the pancreatic juice and the erepsin of the succus entericus. Provided the stomach be left *in situ*, moreover, efficient digestion and absorption of proteins may still continue when the pancreatic duct is ligated, so that pancreatic juice cannot enter the intestine. In this case digestion is effected by erepsin after preliminary cleavage by the pepsin in the gastric juice. The absorption of **Fats**, however, is very seriously interfered with by the exclusion of pancreatic juice from the intestine.

An important relationship subsists between the amount of food which is partaken of at a meal and the quantity of digestive juices secreted on the one hand, and the time occupied in digestion on the other hand. In the case of the **Gastric Juice** the quantity of the digestive fluid secreted may be estimated by forming a diverticulum or pocket in the stomach which is connected with the exterior by means of a fistula. The juice secreted by this diverticulum is found to be a constant proportion of the fluid which is secreted by the whole area of the gastric mucosa and the total secretion of gastric juice during digestion may therefore be estimated in terms of the volume of the secretion furnished by the diverticulum. It has been observed that the quantity of gastric juice which is secreted during the digestion

of a meal is very closely proportionate to the quantity of food of any one kind which is ingested. The following are results obtained by Klugine, the "calculated" figures being estimated on the assumption of strict proportionality between the volume of secretion and the mass of the given type of food which was ingested.

Type of Food.	Quantity of food. grams.	Quantity of gastric juice: Observed. c.c.	Calculated. c.c.
Raw meat	400	106	99
Raw meat	200	41	50
Raw meat	100	27	25
Boiled meat	200	42	42
Boiled meat	100	21	21
Milk	600	56	53
Milk	500	41	44
Milk	200	17	18
Soup of oats and meat	600	43	41
Soup of oats and meat	300	20	21
Meat, bread and milk	800	83	90
Meat, bread and milk	400	41	45

It is evident that the calculated figures agree as closely as could be desired with those actually observed. Evidently, then, there is not a constant amount of gastric juice secreted for each meal, but the amount furnished is proportionate, for any one kind of food, to the mass ingested. As Arrhenius has pointed out, this would appear at first sight to be an uneconomical arrangement, since a very small quantity of a digestive enzyme is capable, in time, of digesting a very great excess of foodstuff. The length of time required for digestion, however, if the mass of enzyme available for each meal were a limited, fixed quantity, would be so extremely variable that the economy of the tissues could not be adjusted to so irregular a method of furnishing their needs. For instance if about four and a half hours are requisite for the gastric digestion of 100 grams of raw meat by a given amount of pepsin then it may readily be calculated from the **Schütz-Borrissov Rule**, which pepsin obeys, that no less than 70 hours would be requisite for the digestion of 400 grams and eighteen days for the digestion of a kilogram. As a matter of fact, however, the process of gastric digestion is carried out in successive portions of the foodstuffs, a fresh supply of gastric juice being furnished for each portion of food that comes into contact with the surface of the gastric mucosa. In this way much more rapid and uniform digestion is secured than would otherwise be possible.

The hydrolysis of foodstuffs in the alimentary canal appears to follow the same quantitative laws as the hydrolyses by the corresponding enzymes *in vitro*. Thus **Gastric Digestion** follows the Schütz-Borrissov rule, while the hydrolysis of protein in the small intestine by **Pancreatic Juice** follows the monomolecular logarithmic formula:

$$\log \frac{a}{a - x} = kt$$

which holds good for the action of this enzyme in glassware.

It is a rather noteworthy fact that the **Rate of Absorption** of digestion-products from the intestine does not appear, in so far as it has been quantitatively investigated, to follow the logarithmic rule, as we should expect if the rate of absorption depended solely upon the concentration, *i. e.*, osmotic pressure, of the substance undergoing absorption. On the contrary, for the absorption of glucose at all events, a square-root rule seems to hold good, *i. e.*, the quantity absorbed in a given time is proportional to the square-root of the concentration of the material which is being absorbed. It is, however, perfectly evident, even apart from these measurements, that the process of absorption cannot be purely a question of the diffusion of substances into and through the wall of the intestine in simple proportion to their osmotic pressures, for otherwise no **Selective Absorption** would be possible. We have seen that certain amino-acids are absorbed preferentially, others being absorbed with relative slowness even when they are present in excess. This implies that besides the forces of osmotic pressure, phenomena of solubility in the absorbing tissue-elements or of chemical affinity therewith play an important or decisive part in determining the relative rates of absorption and the types of material absorbed.

From the **Large Intestine**, as we have seen, the products of bacterial decomposition of foodstuffs may be absorbed, sometimes with physiologically undesirable results. A considerable **Absorption of Water** occurs here also. During digestion and absorption in the stomach and small intestine the contents of the alimentary canal retain a watery consistency which is favorable to the rapidity of hydrolysis, and to the thorough admixture of the digestive secretions with the foodstuffs and the absorption of the products of digestion. In the large intestine, however, a large proportion of this water is absorbed, so that the water-content of the feces is normally considerably less than that of the contents of the small intestine. In case the feces are expelled with undue rapidity, however, and before the absorption of water is complete, as when a cathartic is administered, then the feces have a watery consistency and thirst is engendered through insufficient absorption of the water which has been partaken, and which has also been furnished to the intestinal contents by the various digestive fluids.

Not only water and products of bacterial action may be absorbed from the large intestine, however, but also foodstuffs if they chance to find entry therein without previous absorption. Thus, it is not an uncommon procedure in medical practice to furnish nutrition to very weak individuals or to persons who are unable to swallow, by **Rectal Feeding**, or the introduction of enemas containing fully hydrolyzed foodstuffs, such as glucose. The substances thus administered are found to be absorbed, and to be normally utilized for the maintenance of the tissues, and the provision of energy.

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PART II.

THE PROPERTIES OF PROTOPLASM

CHAPTER XII.

PROPERTIES CONFERRED BY THE DIFFUSIBLE CONSTITUENTS.

THE OSMOTIC PRESSURE OF THE TISSUE FLUIDS.

The diffusible constituents of living matter and of the media which bathe it, play a leading part in determining the movements and distribution of the most abundant constituent of living cells, namely **Water.** Water is a very essential constituent of protoplasm, for a variety of reasons. In the first place it is a solvent for the majority of the protoplasmic constituents, and thus permits their mobility and promotes, by reduction of internal friction and cohesion, the free and rapid interplay of chemical reactions which characterizes the unstable equilibria of life. Then, again, water is the most efficient ionizing solvent, and thus permits electrical forces to come into play, and that notable increase in chemical reactivity which accompanies the ionization of dissolved substances. The low internal friction of water permits the changes of form, and rapid displacements of substance which render the mobility of living matter possible. The high surface-tension of water is essential in the conservation of the boundaries of the cell, and their restoration after displacements due to motion, and this, in turn, conserves the minute internal structures of the cell. The high specific heat of water enables it to absorb a great deal of heat without increasing very greatly in temperature and, conversely, to part with stored-up heat without falling very much in temperature. Sharp inequalities of temperature which might otherwise arise in living tissues are thus smoothed out by the "buffer action" of the prevailing solvent.

It is of interest to consider the percentages of water which are contained in the various tissues of the animal body. The following are illustrative analyses cited after Hammarsten:

Tissue.	Percentage of water
Fatty tissue	6–10
Bone (extremities and skull)	14–22
Bone (vertebræ and ribs)	16–44
Tendon	56–68
Brain, white substance	68–70
Muscular tissue	75–78
Thyroid gland	77–82
Thymus	81
Brain, gray substance	82–85

It will immediately be noted that the percentage of water is highest in those tissues which are undergoing the most rapid metabolic changes and which are called upon to function, in a chemical rather than a structural manner, most rapidly and frequently. The percentage of water is lower in adult than in embryonic tissue, and decreases with advancing age of the tissues, and diminution of the speed of metabolism. Living tissues which are exceptionally poor in water or which withstand dessication, such as seeds or bacterial spores, represent life latent, but arrested, only to be resumed in full vigor upon the readmission of water.

The force which impels the movement of water into or out of the the elements of living matter is the difference between the **Osmotic Pressure** of the fluids within the cell on the one hand, and the external medium which bathes the cell on the other. The manner in which this force may impel the migration of water will be evident if we consider the mechanism by which it originates. We may suppose that the molecules of a substance in solution are in a state of continuous motion, as, indeed, their diffusibility shows that they must be. Let us consider the condition of affairs in a vessel filled with water (Fig. 9) and divided into two parts by a partition *A–B*, on the right-hand side of which we introduce such an amount of some diffusible substance, such as glucose, that there are ten molecules of glucose in the mixture for every ninety molecules of water. Evidently, on the left-hand side of it every molecule which collides with this partition will be a water-molecule, but on the right-hand side every tenth molecule will be a sugar-molecule.

If, now, the partition *A–B* is constructed of such material that it is porous to water, but impermeable for more bulky molecules such as those of sugar, it is evident that out of 100 molecules bombarding the partition from the left all will pass through into the right-hand chamber, while out of 100 molecules bombarding the partition from the right, only 90 will be able to penetrate into the left-hand chamber. In any given interval of time, therefore, an excess of water molecules will have entered into the right-hand chamber, and this excess will be directly proportionate to the concentration of sugar dissolved therein.

Such a partition as that which we have described constitutes what is known as a **Semipermeable Membrane**, and membranes having the characteristic of permitting the passage of water but not of dissolved substances are very numerous. The one most frequently employed

for osmotic-pressure measurements is the membranous precipitate of **Copper Ferrocyanide** which is formed when a solution of copper sulphate comes into contact with a solution of potassium ferrocyanide.

If the continuous entry of water into the right-hand chamber were permitted and the level of fluid did not rise so as to create a pressure, water would pass indefinitely from left to right until the sugar in the right-hand compartment was infinitely diluted. In this way no measurement of the attraction of the solution for water would be possible, since, in theory, if no frictional forces or pressures interfered with the free motion of water, every solution, concentrated or dilute, would attract an infinite volume of water. We may, however, measure the degree of attraction for water which is exerted by the dissolved substance by determining the pressure or temperature necessary to

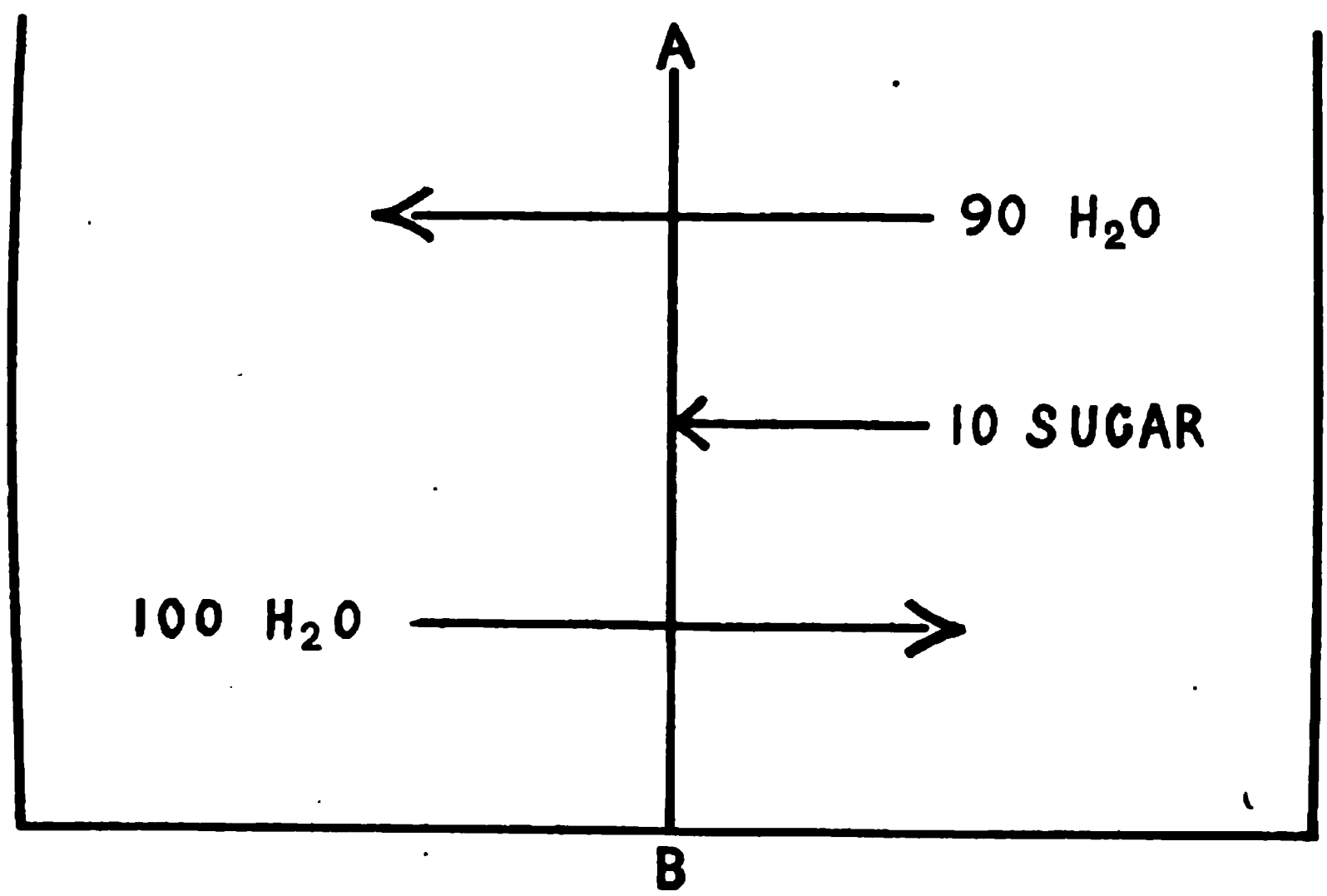

FIG. 9

increase the force or frequency of the impacts on the right-hand side of the partition, until the greater *speed* of transit from right to left compensates for the greater *volume* of transit from left to right. If pressure be applied to the contents of the right-hand compartment, the force of the impacts of the molecules upon the partition is increased so that although only ninety water molecules collide with the right-hand side of the partition for every hundred which collide with the left-hand side, yet those colliding on the right do so more forcibly, and thus a greater proportion succeed in penetrating the membrane, until, when the pressure applied to the solution attains a certain magnitude the greater proportion of collisions leading to penetration of the membrane exactly balances the excess of the total number of collisions on the side which is bathed by pure water.

The pressure which is required to exactly compensate the greater frequency of collisions on the side of the membrane which is bathed by pure water is termed the **Osmotic Pressure** of the solution. Its measurement may be rendered automatic by enclosing the solution in a vessel to the orifice of which a **Manometer** is attached, so that the entry of a very minute amount of water, into the vessel, insufficient to appreciably dilute the solution, causes a very considerable rise in the mercury column of the manometer, and a proportionately large increase of pressure. With practically negligible dilution of the solution, therefore, the necessary pressure is attained and cannot be exceeded, because the os notic pressure having once been attained, the rates of entry and exit of water into and o ıt of the vessel become equal and no further changes of pressure or composition can occur.

Such a vessel, provided with a semipermeable membrane and a manometer, is termed an **Osmometer.** Since the pressures which are generated are usually very great, the walls of the vessel must be made of strong material, and the membrane, especially, must be constructed so as ıot to break under the strain. These desiderata are attained by employing a vessel composed of earthenware, in the minute pores of which membranes are formed by filling the vessel with a solution of potassium ferrocyanide and immersing it in a solution of copper sulphate. The two reagents diffuse outward and inward, or may be induced to do so by electrolysis, until they meet at some point within the pores across which membranes of minute dia ıeter are formed. Such membranes withstand relatively enormous pressures, while a large continuous membrane would rupture under the strain of much smaller pressures.

Another way of equalizing the rates of transposition of water across the membrane would be to raise the **Temperature** of the solution above that of the water upon the other side of the membrane. Increase in temperature results in proportionate increase of the mobility of the molecules, so that the collisions with the membrane would be proportionately more numerous per unit of time on the heated than on the unheated side. This procedure is not practicable with the membranes which we have hitherto been considering, because they would conduct heat from the one chamber to the other and the temperatures of the two compartments on either side of the membrane would soon be equalized. We may very easily employ this method, however, if in the place of a thin solid membrane we employ a layer of air. Then, provided the dissolved substance is not volatile, *i. e.*, soluble in air, we have in effect a semipermeable membrane which is a poor conductor of heat and which may be obtained of any thickness which we may desire. If two chambers or vessels, the one containing water, the other containing a solution, be both placed in a confined space or large vessel filled with air, water will, if the two liquids are at the same temperature, slowly distil over from the compart nent co ıtaining pure water into the compartment containing the solution, which thus becomes pro-

gressively more dilute. If unchecked by any balancing or opposing influence, this distillation will continue until the solution becomes infinitely dilute, *i. e.*, practically equivalent to distilled water. In this case, therefore, as in the case of thin solid membranes, we can only measure the attraction of the solution for water by measuring the change in the condition of the solution requisite for its neutralization. This we may accomplish by heating the solution and thus increasing the mobility of the molecules which it contains, and so increasing the number of collisions per second of water-molecules with the supernatant layer of air. The temperature to which we must raise the solution in order to equalize the rates of distillation to and from the water and the solution is proportionate to the increase in the collisions per second which is requisite to produce this equalization, and this in turn must obviously be proportionate to the concentration of the dissolved substance. Thus if the dissolved substance constitutes one-tenth of the total molecules in the solution, we must raise the temperature of the solution sufficiently to increase the total collisions per second by one-tenth, in order to render the rate of distillation equal to that of pure water at the lower temperature. If this rate of distillation is sufficient to cause ebullition, *i. e.*, to render the pressure of water-vapor equal to that of the atmosphere, it is evident that the temperature required to attain it will be higher in the case of the solution than in the case of pure water. Hence, the **Boiling-point** of water is raised by dissolved substances, and that in proportion to their molecular concentration.

There is yet another way in which we may equalize the rates of penetration of water from opposite sides of the membrane, and that is by *cooling* the water, and thus reducing the mobility of its molecules relatively to those of the solution. Now when a solution freezes, it is not the dissolved substance that freezes, but the solvent, in this case water. The dissolved substance, in fact, with certain intelligible exceptions, crystallizes out and becomes mechanically separated from the solvent when the latter freezes. In such a case the membrane is furnished by the surface separating the crystals of ice from the remainder of the solution. If, now, reverting to the diagram in Fig. 9, the water in the left-hand compartment be sufficiently cooled, relatively to the solution, water will pass over from the warm solution into the cool chamber of pure water, because of the greater mobility of the molecules in the warm solution.[1] Hence, in order to accomplish the withdrawal of water from the dissolved substance which occurs at the freezing-point the water must be cooled to a temperature

[1] Ultimately, however, the greater mobility of the molecules in the solution will fail to compensate for the progressively decreasing proportion of water molecules present in the solution, and the water compartment would have to be further cooled in order to continue withdrawal of water from the solution. This is the phenomenon of "undercooling" which freezing salt solutions display. The correct freezing-point is that at which the first crystal of ice separates, and which is marked by a sudden slight rise of temperature due to the disengagement of the latent heat of fusion of the ice.

below the freezing-point of pure water. Hence, the **Freezing-point** of water is lowered by dissolved substances, and that in proportion to their molecular concentration.

The osmotic pressure of a solution of a diffusible substance may therefore be measured either directly, employing a semipermeable membrane, or indirectly, by measuring the elevation of the boiling-point or the lowering of the freezing-point. Conversely the molecular concentration of a dissolved substance may be estimated in the same ways. The osmotic pressure exerted by a molecular solution, that is, by one gram-molecule of substance dissolved in a liter of water is 22.4 atmospheres. The elevation of the boiling-point in the same solution is 0.54°, while the depression of the freezing-point is 1.86°. If, however, the dissolved substance undergoes **Electrolytic Dissociation** then each of the ions which it yields exerts osmotic pressure and affects the boiling- and freezing-points in the same way as a molecule, so that for a substance *completely* dissociated into two ions, such as sodium chloride in dilute solution, the osmotic pressure per gram-molecule of dissolved substance is double the above-mentioned figure, and the molecular elevation of the boiling-point and lowering of the freezing-point are similarly enhanced. If different solvents are employed the osmotic pressures obtained are the same as those obtained when water is used as a solvent, provided the molecular condition of the dissolved substance is the same in both solvents, but if it be ionized in one and not in the other, or forms double molecules in one and not in the other solvent, the pressures observed will, of course, differ from one another in a corresponding manner and degree. The magnitude of the effect upon the boiling- and freezing-points, although always proportionate in any one solvent to the molecular *plus* ionic concentration of the dissolved substance, differs with different solvents.

The osmotic pressures of tissue-fluids and of fluids expressed from cells are usually estimated by the **Cryoscopic Method** or measurement of the lowering of the freezing-point of the solvent, in this case water. This measurement is much less tedious and less subject to interference by colloidal admixtures, etc., than the direct measurement of pressure in an osmometer. The elevation of the boiling-point is usually not applicable because of the extensive changes induced in these solutions by elevated temperatures, for example the coagulation of proteins and the transformation of bicarbonates into carbonates with the evolution of carbon dioxide. The former of these changes can be obviated, of course, by measuring the elevation of the boiling-point under reduced pressures when ebullition occurs at a correspondingly lower temperature, but the difficulty created by the evolution of carbon dioxide still remains.

The following are illustrative measurements, obtained by Hamburger and others, of the lowering of the freezing-point in blood-sera of various species of *Mammalia:*

Species.	Lowering of freezing-point.
Man	0.526°
Ox	0.585°
Horse	0.564°
Pig	0.615°
Rabbit	0.592°
Dog	0.571°
Cat	0.638°
Sheep	0.619°
Echidna hystrix	0.600°

From this table two remarkable facts are apparent: In the first place that the osmotic pressure of the blood of species of mammalia so diverse as man, herbivora, carnivora and the monotremes is extraordinarily constant, and in the second place that it has the magnitude of no less than some eight atmospheres, corresponding to the pressure exerted by a one-third molecular solution of a non-ionized substance such as sugar or urea, or a one-sixth molecular solution of sodium chloride.

The osmotic pressure of the blood-serum, as evaluated from the lowering of the freezing-point, rises slightly, but unmistakably, after the absorption of the products of digestion derived from the meal. The **Lymph** has usually a higher osmotic pressure than the blood, a fact which is attributed to the extraction of products of metabolic activity from the tissues somewhat more rapidly than they can be discharged from the lymph into the blood. **Milk** and **Bile** have the same osmotic pressure as blood, **Saliva** a lower pressure. **Urine** is in general much more concentrated in diffusible constituents than the blood or tissue-fluids and therefore displays a much greater lowering of the freezing-point, usually between 1.3° and 2.3°.

The blood-sera of **Birds** possess an osmotic pressure very similar to that of mammalian blood-sera. It is a curious fact, however, that the **Eggs** of birds have a distinctly lower osmotic pressure than that of the blood-serum of the birds that lay them, or of the blood-serum of the embryos that develop within them. This is strikingly shown by the following estimations of Atkins.

Species.	Lowering of freezing-point.
Fowl-blood	0.607°
Fowl-egg	0.454°
Duck-blood	0.574°
Duck-egg	0.452°
Goose-blood	0.552°
Goose-egg	0.420°

During **Incubation** of the egg the osmotic pressure of its contents increases until it approximates to that of the blood. Since in so many anatomical particulars the **Ontogeny** of the individual represents an abbreviated outline of the **Phylogeny** of the species, Atkins has suggested that the low osmotic pressure of the egg-contents may indicate descent of the birds from ancestors in which the blood-serum was more dilute than it is in the birds of the present epoch. Since the birds

are probably descended from ancient forms of *Reptilia* or from forms intermediate between the *Reptilia* and the *Amphibia* it is of interest to note that many of the *Amphibia* and some of the *Reptilia* which inhabit fresh water exhibit a low osmotic pressure of the blood-serum. The following are illustrative figures cited after Höber and Jona.

Species.	Lowering of freezing-point.
Amphibia:	
Rana esculenta	0.465°
Salamandra maculata	0.479°
Reptilia:	
Emys europaia	0.474°
Emydura macquariæ	0.550°
Thalassochelys caretta	0.610°

It will be observed that the fresh-water turtle, *Emys europaia*, has blood of which the osmotic pressure approaches the amphibian type, the marine turtle, *Thalassochelys caretta* has the avian and mammalian type of osmotic pressure of the blood, while the tortoise, *Emydura macquariæ* represents an intermediate pressure. The osmotic pressure of amphibian blood-serum closely approaches in magnitude the pressures obtaining in the eggs of birds.

Among the various orders of **Fishes** in the *Teleostomi* or bony fishes, which are phylogenetically the most recent and highly developed forms, the osmotic pressure of the blood-serum approximates much more nearly to that of the blood of *Mammalia*, *Aves* and *Reptilia* than to the osmotic pressure of the ocean which the marine forms inhabit. In the phylogenetically older and less specialized forms, the *Elasmobranchii* or sharks, however, the osmotic pressure of the blood approximates that of sea-water, as the following figures, cited after Bottazzi, reveal:

Fluid.	Lowering of freezing-point.
Sea-water	2.30°
Elasmobranchii:	
Torpedo marmorata	2.26°
Mustelus vulgaris	2.36°
Trygon violacea	2.44°
Marine teleostomi:	
Charax puntazzo	1.04°
Cerna gigas	1.04°
Crenilabrus pavo	0.75°
Box salpa	0.84°
Fresh-water teleostomi:	
Anguilla vulgaris	0.58°–0.69°
Barbus fluviatilis	0.475°–0.558°
Leuciscus dobula	0.45°
Perca fluviatilis, *Cyprinus carpio*, *Tinca vulgaris*, *Esox lucius*	0.51°

In lower marine forms the tissue-fluids approximate still more closely in composition and concentration to the sea-water which these organisms inhabit. The following are results obtained by Bottazzi:

Organism.	Lowering of freezing-point of tissue-fluids.
Cœlenterata:	
Alcyonium palmatum	2.196°
Echinodermata:	
Asteropecten aurantiacus	2.312°
Holothuria tubulosa	2.315°
Vermes:	
Sipunculus nudus	2.31°
Crustacea:	
Maja squinado	2.36°
Homarus vulgaris	2.29°
Cephalopoda:	
Octopus macropus	2.24°

With the enhanced specialization, therefore, which characterizes the higher and especially the vertebrate forms of life, independence of the external milieu has been acquired and the cells are bathed in a medium of relatively constant concentration and, as we shall see, of even more constant composition.

THE OSMOTIC PRESSURE OF CELL-CONTENTS.

The osmotic pressure of cell-contents can, of course, be determined indirectly by expressing the cell-sap and determining its freezing-point. In many cases, however, the measurement may be made in a very much more convenient manner by employing the method of **Plasmolysis** devised in 1884 by the Dutch botanist, de Vries.

In many plants the protoplasm of the cells lies closely adherent to the cellulose cell-wall, and it is found if these cells be immersed in concentrated solutions of salts, sugars, urea or other diffusible substances, that the protoplasm shrinks away from the supporting wall of cellulose, indicating that the protoplasm has diminished in volume. This loss of volume can only be due to the abstraction of water from the protoplasm, and since the agencies which accomplish this abstraction of water are solutions of relatively high osmotic pressures, we infer that the external limiting membranes of the cells, within the cellulose cell-wall but bounding the exterior of the protoplasmic contents, is **Semipermeable**, admitting water but not admitting a variety of diffusible dissolved substances.

If this interpretation be the correct one, then any solution having a higher osmotic pressure than the cell-fluids will cause plasmolysis, while the solutions which are of just the same osmotic pressure as the cellular fluid will fail to cause plasmolysis. The solutions which just fail to cause plasmolysis, or which are **Isotonic** with the cell-fluids, should therefore all be of the same molecular or molecular plus ionic concentration, independently of the nature of the dissolved substance, provided, only, that it is not able to penetrate the cell-membrane in measurable proportion within the period of time consumed by the shrinkage of the protoplasm.

The following are results which were obtained by Overton, employing the cells of *spirogyra* filaments:

Dissolved substance.	Molecular weight.	Isotonic concentration: found, per cent.	Isotonic concentration: calculated, per cent.
Cane-sugar	342	6.0	..
Mannitol	182	3.5	3.19
Glucose	180	3.3	3.15
Arabinose	150	2.7	2.63
Erythritol	122	2.3	2.14
Asparagin	132	2.5	2.32
Glycocoll	75	1.3	1.32

The "calculated" values were computed as follows: The isotonic concentration of cane-sugar being 6 per cent. and its molecular weight 342, the concentration of an isotonic solution is evidently $\frac{60}{342} = \frac{1}{5.7}$ molecular. A $\frac{1}{5.7}$ molecular solution of glycocoll would contain $\frac{75}{5.7}$ grams of glycocoll per liter, or 1.32 grams per hundred c.c. It will be seen that the experimental and the calculated values are exceedingly close to one another and we may infer that, at all events so far as limited periods of time are concerned, the protoplasm of *spirogyra* is impermeable to the substances mentioned, although freely permeable to water.

This method of estimating the isotonicity of solutions, however, is subject to several sources of error and uncertainty. In the first place we must take into consideration the fact that the protoplasmic limiting membrane must necessarily alter in form before we can perceive any solution to be **Hypertonic**, or in excess of the isotonic concentration. Now the external limiting membranes of cells must undoubtedly possess some degree of **Elasticity**, in consequence of which they must themselves exert some pressure upon the cell-contents. The forces leading to shrinkage of the protoplasm are not solely osmotic therefore, but to some slight extent elastic also, and we cannot positively estimate the proportion of the total force which this elasticity communicates, since it will not improbably add a constant amount to each osmotic pressure investigated. Isotonic solutions are therefore isosmotic with one another, but not necessarily isosmotic with the cell-contents. In red blood-corpuscles this is probably the origin of the constant slight difference between the osmotic concentration of the contents of the corpuscles and the surrounding medium or plasma, amounting, according to Moore and Roaf, to a difference of freezing-point depression of 0.02° to 0.03° C., or an osmotic-pressure difference of from 0.24 to 0.36 of an atmosphere.

In the second place, the **Semipermeability** of living cell-membranes is, of necessity, never absolute. This becomes obvious when we consider that the nutrition, and therefore, the maintenance and growth of cells depends upon their intake of substances dissolved in water. Unless a cell can be penetrated by the mineral or organic substances which constitute the components out of which protoplasm is built up, the progressive consumption of material and dissipation

of energy by the cell must rapidly lead to its disintegration. Furthermore, the solutions which Overton, in the results cited above, found to be isotonic with the cell-contents of spirogyra filaments exerted an osmotic pressure of some four and a half atmospheres, and the corresponding pressure in the cell-contents themselves, can only have been due to diffusible water-soluble substances which must therefore have penetrated the protoplasm at some period of its development. The semipermeability of cell-membranes is in fact, even in the most typical instances, apparent and not real. It is purely a question of **Relative Permeability**, of the *rapidity* with which dissolved substances and water relatively penetrate the cell. In the case of *Bacillus choleræ*, for example, the relativity of the semipermeability of cells can very clearly be seen, for these organisms, as well as certain other bacteria, are *temporarily* plasmolyzed by hypertonic salt solutions or sugar-solutions, but not at all by **Glycerol** solutions. Even the plasmolysis observed in salt- or sugar-solutions disappears in the course of an hour or two, because, after the lapse of this time, a sufficient proportion of the salt or sugar has penetrated the cells to restore isotonicity between the inner and outer fluids. Evidently, therefore, in the case of these cells water and glycerol penetrate the exterior limiting membrane almost instantaneously, salt and sugar more slowly. The disparity of the velocities of penetration for water and dissolved substances is greater in *spirogyra* filaments than in the above-mentioned species of bacteria, and this constitutes the origin of the apparent semipermeability of the protoplasm in *spirogyra*.

It is a rather remarkable, and certainly a regrettable fact that physical chemists have hitherto paid so little attention to the investigation of the **Time-relation of Osmosis**. The comparative neglect of this and other fields of inquiry which would naturally suggest themselves to the student of pure physics or mechanics, is undoubtedly attributable to the bias toward purely thermodynamical reasoning which has been communicated to the students of physical chemistry by the past generation of chemists. The thermodynamical relationships and equations contemplate only attained **equilibria**, not fluctuating or kinetic phenomena. Hence, the relationship between the lapse of time and the degree of penetration of a membrane by various solvents or dissolved substances, which would seem to present a most obvious subject for inquiry, is as yet very imperfectly known. One would expect, however, that the quantity of penetration would be an exponential function of the time, and that this function would contain specific parameters or constants, characteristic for the membrane, the particular solvent employed, and the dissolved substance respectively. The evaluation of these parameters in the case of living cell-membranes would afford an accurate quantitative measure of **Permeability**, for the estimation of which we must rely at present upon qualitative rather than upon quantitative data.

In the plasmolytic method of estimating isotonic solutions we regard

as isotonic those solutions which are just insufficiently **Hypertonic** to cause withdrawal of water from the cell-contents. The effects of **Hypotonic** solutions or solutions which are more dilute than the cell-content are more readily studied in cells which possess no rigid supporting framework, such as the exterior cellulose wall of plant-cells. The **Red Blood-corpuscles** were first employed by Hamburger for this purpose. If these cells are suspended in sufficiently hypotonic solutions, the excessive penetration of water into the cells results in their rupture by the internal pressure which results, and hemoglobin is set free, tingeing the supernatant fluid red. The technique, therefore, consists in suspending the corpuscles in solutions of varying concentration and allowing them to settle to the bottom of the tube. A solution which is just sufficiently hypotonic to burst some of the corpuscles will be tinged with hemoglobin and the corpuscles are then said to have undergone **Hemolysis.**

The degree of hypotonicity required to rupture red blood-corpuscles is apparently the same for a variety of dissolved substances, so that the solutions are found to be isotonic with one another, as the following data show:

Substance.	Molecular weight.	Limiting concentration which causes hemolysis, per cent.
NaCl	58.5	0.585
CH_3COOK	98.1	1.04
KNO_3	101.1	1.00
NaBr	102.9	1.02
NaI	149.9	1.55
KI	166.0	1.65

Solutions which cause hemolysis, although isotonic with one another are, of course, by no means isotonic with the fluid contents of the corpuscles, for the bursting of the cells indicates not a slight but a very considerable excess of pressure within them. Solutions insufficiently hypotonic to cause actual rupture of the cells will nevertheless cause them to swell through absorption of water, while slightly hypertonic solutions will, on the contrary, cause shrinkage of the cells through the withdrawal of water, just as in the plasmolysis of plant-cells. This is the foundation of the **Hematocrit** method of measuring isotonicity, devised by Hedin and Koeppe. Blood-corpuscles, freed from serum by washing them with isotonic salt solution, are suspended in measured amounts of various solutions to be investigated, and the mixtures are placed in specially constructed centrifuge-tubes of very narrow bore and provided with fine graduations. The tubes are then centrifuged and the heights of the columns of corpuscles compared in the various tubes. If the corpuscles have swollen they will occupy a larger volume in the tube, if they have lost water they will occupy a smaller volume than the corpuscles which are immersed in strictly isotonic salt solutions. From the lowering of the freezing-point we know that blood-serum is isotonic with $\frac{m}{6}$ NaCl or $\frac{m}{3}$ sugar solutions, and it is experimentally found that in the majority of instances salt solutions which

slightly exceed this concentration cause shrinkage of the corpuscles, while solutions which are less concentrated than blood-serum cause swelling of the corpuscles.

Assuming the corpuscles in normal serum to be withstanding no pressure or, at the most, a very slight one, it is of some interest to calculate from the degree of hypotonicity the pressure which is required to rupture the corpuscles so as to discharge hemoglobin into the solution. The solutions which are just sufficiently concentrated to prevent rupture are, as we have seen, isotonic with a one-tenth molecular sodium chloride solution or, which comes in terms of osmotic pressure to the same thing, a one-fifth molecular solution of sugar. When neither swollen nor shrunken these cells are isotonic with a one third molecular solution of sugar. The degree of hypotonicity required to rupture the cells therefore, corresponds to the pressure exerted by a $\frac{1}{3} - \frac{1}{5} = \frac{2}{15}$ molecular solution of sugar, *i. e.*, to a pressure of about three atmospheres.

From the data which we have cited the surface of a red blood-corpuscle would appear to afford an example of a strictly semipermeable membrane. Here again, however, semipermeability is relative and not absolute. Not only water can enter the cells with ease but also other substances with varying difficulty. An ingenious method of illustrating this fact is that which has been devised by Hedin.

A measured amount of the substance for which the permeability of the corpuscles is to be tested is dissolved in defibrinated blood, *i. e.*, in a mixture of serum and corpuscles. The serum of this blood will be found to freeze at a lower temperature than untreated serum, because a certain proportion of an additional diffusible substance is contained in it. The depression of the freezing-point of this serum may be designated "a." Now to an equal volume of serum which does not contain any corpuscles an equal amount of the same substance is added. This serum will also freeze at a lower temperature than normal serum, and the depression of the freezing-point which it exhibits may be designated "b." Now, it is evident that if the substance which was added to the defibrinated blood penetrated the corpuscles and dissolved in them to the same extent as in an equal volume of serum, the concentrations of the substance in the two samples of serum would be equal to one another, and we would have $a = b$. If the blood corpuscles in the defibrinated blood took up less of the dissolved substance than an equal volume of serum, then the substance would be present in greater concentration in the first sample of serum than in the second, and we would have $a > b$ or $\frac{a}{b} > 1$. If, on the other hand, the blood-corpuscles took up more of the dissolved substance than an equal volume of serum then we would have $a < b$ or $\frac{a}{b} < 1$.

The results of this method show that the salts of the alkalies and alkaline earths and the amino-acids and sugars penetrate the corpuscles with great difficulty. **Ammonium Salts** and **Urea**, however, pass into the

corpuscles readily. Among the alcohols there is an interesting relationship between the number of hydroxyl-groups which they contain and the readiness with which they penetrate the corpuscles. The hexatomic and pentatomic alcohols hardly penetrate the corpuscles at all. **Erythritol**, which is a tetra-atomic alcohol and **Glycerol** which is triatomic penetrate slowly. **Ethylene Glycol**, which is a diatomic alcohol, penetrates the cells rather rapidly, while the **Monatomic Alchols** divide themselves immediately in equal proportion between the corpuscles and the serum. Ether, esters, aldehyde and acetone, on the other hand, are preferentially absorbed by the corpuscles, so that they become more concentrated in the corpuscles than in the serum which bathes them. Of course only those substances which fail to enter the cells quickly can cause shrinkage of the corpuscles in hypertonic solutions.

THE COMPOSITION OF THE MINERAL CONSTITUENTS OF TISSUE FLUIDS.

It was first pointed out by Ringer in 1882 that the relative proportions of the mineral constituents in the blood-sera of different mammals are most remarkably constant and, furthermore, that notwithstanding the fact that potassium and calcium salts are present in blood-serum only in minute proportion relatively to the sodium salts, yet their presence in the established proportion is actually essential to the proper performance of their functions by the tissues, a very slight alteration in the mineral composition of the fluid bathing them being very deleterious.

On the basis of numerous analyses of the ash of blood-sera, the following composition was established by Locke as the most suitable circulating fluid for mammalian tissues: NaCl, 0.9 per cent.; KCl, 0.042 per cent.; $CaCl_2$, 0.024 per cent. To this mixture a small proportion (0.01 to 0.03 per cent.) of sodium bicarbonate is generally added to neutralize the acids which are produced by tissue-activities and a little glucose (0.1 per cent.) has been shown to prolong the life of excised tissues which are kept for prolonged periods in this artificial circulating fluid. The glucose is consumed by the tissue and probably serves as a nutrient. When the glucose is omitted this mixture is usually designated **Ringer's Solution.**

For amphibian tissues a slightly more dilute solution is employed. The solution originally recommended by Ringer was a 0.6 per cent. solution of sodium chloride saturated with calcium phosphate to which 0.03 per cent. of potassium chloride was added. A suitable fluid may also be prepared by simply adding to **Locke's Solution** one-third of its volume of distilled water. Loeb has pointed out that the proportions of the various salts in Ringer's and Locke's solutions correspond approximately to the ratios: 100 molecules of NaCl to 2 molecules

of KCl to 2 molecules of $CaCl_2$, the total concentration being one-sixth molecular.[1]

Not only, however, are the mineral constituents of mammalian serum constant in composition, but even in the blood of fishes we find that substantially the same relative proportions obtain. The following analyses are cited after Macallum, the *percentage*-concentration of sodium being taken as 100 and the percentages of the remaining metals reduced to the same units:

Species.	Na	K	Ca	Mg
Dogfish (*Acanthias vulgaris*)	100	4.6	2.7	2.5
Cod (*Gadus callarias*)	100	9.5	3.9	1.4
Pollock (*Pollachius virens*)	100	4.3	3.1	1.5
Dog	100	6.9	2.5	0.8
Mammal (average)	100	6.7	2.6	0.8
Man	100	6.1	2.7	0.9

The remarkable uniformity of composition which is thus displayed by the blood-sera of such diverse organisms, suggests that it is determined by some common cause, more especially since a slight alteration of the normal mineral composition of blood-sera causes profound disturbance of the functions of the tissues. The interesting suggestion has been put forward by Macallum that the mineral composition of vertebrate blood-sera represents the composition of the sea-water at the time when the early ancestors of the present vertebrate forms first acquired an organ, namely the kidney, of which the function is to maintain constancy of composition in the body-fluids. In the lower marine forms of the present day which do not possess any corresponding excretory organ, the composition and concentration of the body-fluids is practically identical with that of the sea-water in which they live, but in mammals the tissue-fluids are not only more dilute than present-day sea-water, but they differ from it in containing a much smaller proportion of one mineral constituent, namely **Magnesium**. The following figures are cited after Macallum, the *percentage*-concentration of sodium, as before, being taken as 100 and the percentages of the remaining metals reduced to the same standard.

Fluid.	Na	K	Ca	Mg
Ocean-water	100	3.6	3.9	12.1
Tissue-fluid of a jellyfish (*Aurelia flavidula*)	100	5.2	4.1	11.4
Blood-serum of a dog	100	6.9	2.5	0.8

The correspondence of the three sets of figures, excepting in regard to magnesium, is certainly striking and the oceanic origin of these widely-found ratios appears very probable.

Among the crustaceans the more primitive forms, such as *Limulus* possess a blood-serum which is practically of the same composition as sea-water. In more highly developed forms such as *Homarus* an

[1] The actual ratios in Locke's solution are 100 NaCl : 3.6 KCl : 1.4 $CaCl_2$. In sea-water the ratios are: 100 NaCl : 2.2 KCl : 1.5 $CaCl_2$.

approach toward the vertebrate composition of the serum is already indicated, as the following figures reveal:

Fluid.	Na	K	Ca	Mg
Ocean-water	100	3.6	3.9	12.1
Serum of *Limulus polyphemus*	100	5.6	4.1	11.2
Serum of *Homarus americanus*	100	3.7	4.9	1.7

According to Macallum the development of a kidney in the proto-vertebrate forms from which vertebrates have arisen, fixed the composition of the tissue-fluids of the vertebrata for all time, since the primitive kidney was adapted to the concentration and proportions of the mineral constituents of the ocean of that period. In the early Cambrian or pre-Cambrian period at which the ancestral forms of the vertebrates arose, the sea-water must have been very much more dilute than it is at present day, because sodium chloride is constantly accumulating, since it is not deposited in important amounts in the marine geological formations. Calcium and potassium are deposited from sea-water in the form of limestone and minerals such as *glauconite* at about the same rate as that at which they are carried into the sea by rivers. Magnesium, however, is increasing in the sea-water not only absolutely but also relatively to the sodium, the rate of deposition being much slower than the rate of addition. It is quite probable, therefore, that the sea-water of the early Cambrian epoch was not only much more dilute than the sea-water of our day, but also contained both absolutely and relatively much less magnesium.

The blood-serum of mammals therefore resembles a diluted sea-water with the exception that its magnesium content is both absolutely and relatively much lower than the magnesium content of the sea-water of our own day. Just as the homoiothermal animals have acquired a large measure of independence of the temperature of their environment, so, and at an earlier stage of evolution, the vertebrates have acquired a large measure of independence of their osmotic environment,—they are "homoiosmotic," while the more elementary forms are "poikilosmotic" and the cells of which they are composed are exposed to all the disadvantages of an irregularly fluctuating milieu. At a still earlier stage of evolution the multicellular organisms acquired, as we shall see, more or less efficient means of maintaining constancy of the reaction or hydrogen ion concentration of their tissue-fluids. Each of these successive stages marked an additional degree of emancipation from the fortuitous inequalities of an unstable environment and a step toward the self-creation of an equable "internal environment," suitable for the maximum furtherance of vital activities.

The mechanism by which this environmental stability is brought about is similar in each of the three cases and consists in a balance between income and output so adjusted that the dissipating agencies (excretory activity of the kidneys, radiation of heat from the surface of the body, and release of carbon dioxide from the lungs, respectively)

discharge their functions under the stimulus of a definite positive or negative pressure, acting like so many dams, to maintain the reservoir of mineral constituents, heat, or bases at a certain height while the inflow and outflow are equalized so that the height of the reservoir does not progressively increase or decrease. We have in fact in each case a number of balanced activities in dynamic equilibrium, a type of mechanism which is repeatedly reduplicated in life-phenomena.

Notwithstanding the fact that the mineral composition of mammalian blood-sera differs appreciably from that of sea-water only in total concentration and in the relative content of a single constituent, **Magnesium**, yet this latter difference renders sea-water, even when diluted to isotonicity with blood-serum, far from a physiologically neutral fluid for mammalian tissues. It has been shown by Burnett that sterilized sea-water, rendered isotonic with blood-serum by dilution, causes **Glycosuria**, considerable amounts of glucose appearing in the urine when the sea-water is injected into the circulation of rabbits. The same effect is brought about by Locke's or Ringer's solutions, if magnesium is added to them in the proportion in which it is present in sea-water. Hence diluted and sterilized sea-water cannot be employed for surgical purposes as a substitute for Locke's or Ringer's solution.

THE NEUTRALITY OF THE TISSUES AND TISSUE-FLUIDS.

The statements concerning the alkalinity of the blood which are to be found in the physiological and medical literature of the last and early part of this century are totally unreliable since they were based upon the erroneous belief that it is possible to ascertain the reaction of such a fluid as the blood by titration. The method of titration merely informs us of the quantity of bases which are present either uncombined or else combined with weak acids such as carbonic acid, which are displaced from their compounds by the stronger acids used in titration. If all of the bases are present in the free, uncombined form then, in dilute solutions at all events, the true alkalinity or hydroxyl ion concentration may be fairly accurately estimated to be equivalent to the amount of acid required for neutralization. But this is not at all the case if the bases are partially or wholly combined with weak acids, because in that event the addition of the acid employed in titration displaces the weak acid which, when uncombined, by reason of its slight dissociability, ceases to affect materially the reaction of the fluid, and the condition which we are seeking to measure alters continuously throughout the titration. Thus it is possible to estimate all of the sodium in a solution of sodium bicarbonate by direct titration with sulphuric acid, using **Methyl Orange** as an indicator, because the carbon dioxide which is displaced by the sulphuric acid, is so slightly dissociated in comparison with the acid used for titration that its contribution to the final reaction of the mixture is negligible. Yet a

solution of sodium bicarbonate is far from possessing the alkalinity of a solution of free sodium hydroxide of the same concentration, although so far as the results of titration reveal there is no distinction between them.

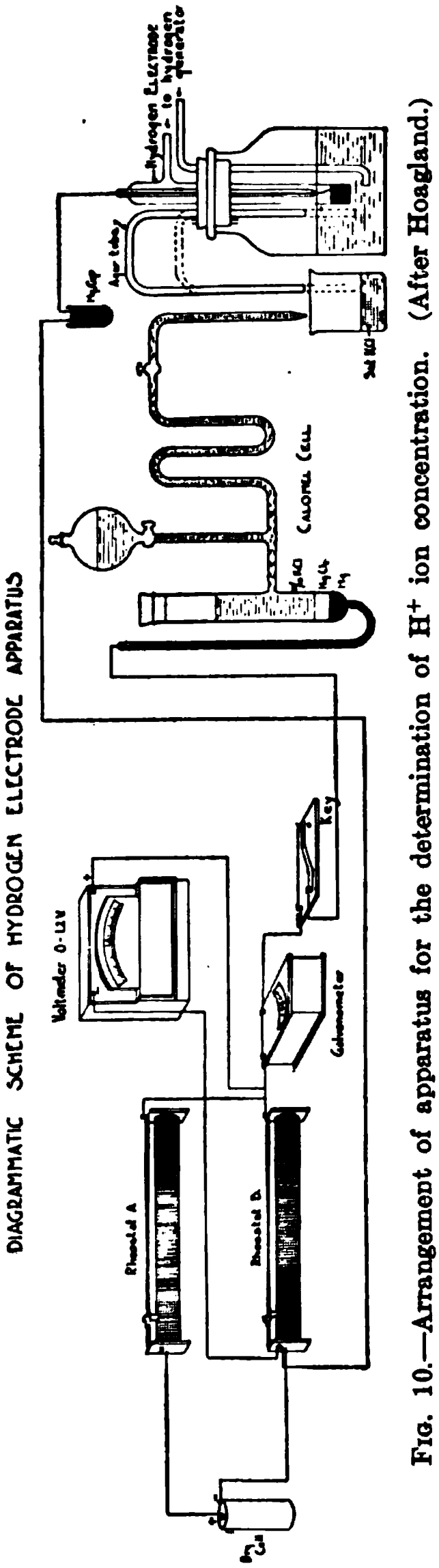

Fig. 10.—Arrangement of apparatus for the determination of H^+ ion concentration. (After Hoagland.)

The blood and other tissue-fluids contain a large proportion of the sodium salts of weak acids, namely carbonic acid, phosphoric acid and proteins. When blood-serum is titrated with hydrochloric or sulphuric

acid, using methyl orange as an indicator, by the time the red color appears these compounds have been successively decomposed, and, in fact, some proportion of the acid employed in the titration has actually entered into combination with the proteins which are now acting as bases instead of acting, as they do in normal blood, as weak acids. The **Titratable Alkalinity** of the blood, therefore, bears no relationship to its actual alkalinity or **Hydroxyl ion Concentration.** It does, however, bear some relationship, as we shall see to the power of the blood to maintain its neutrality, in other words to the "**Alkali-reserve**" of blood.

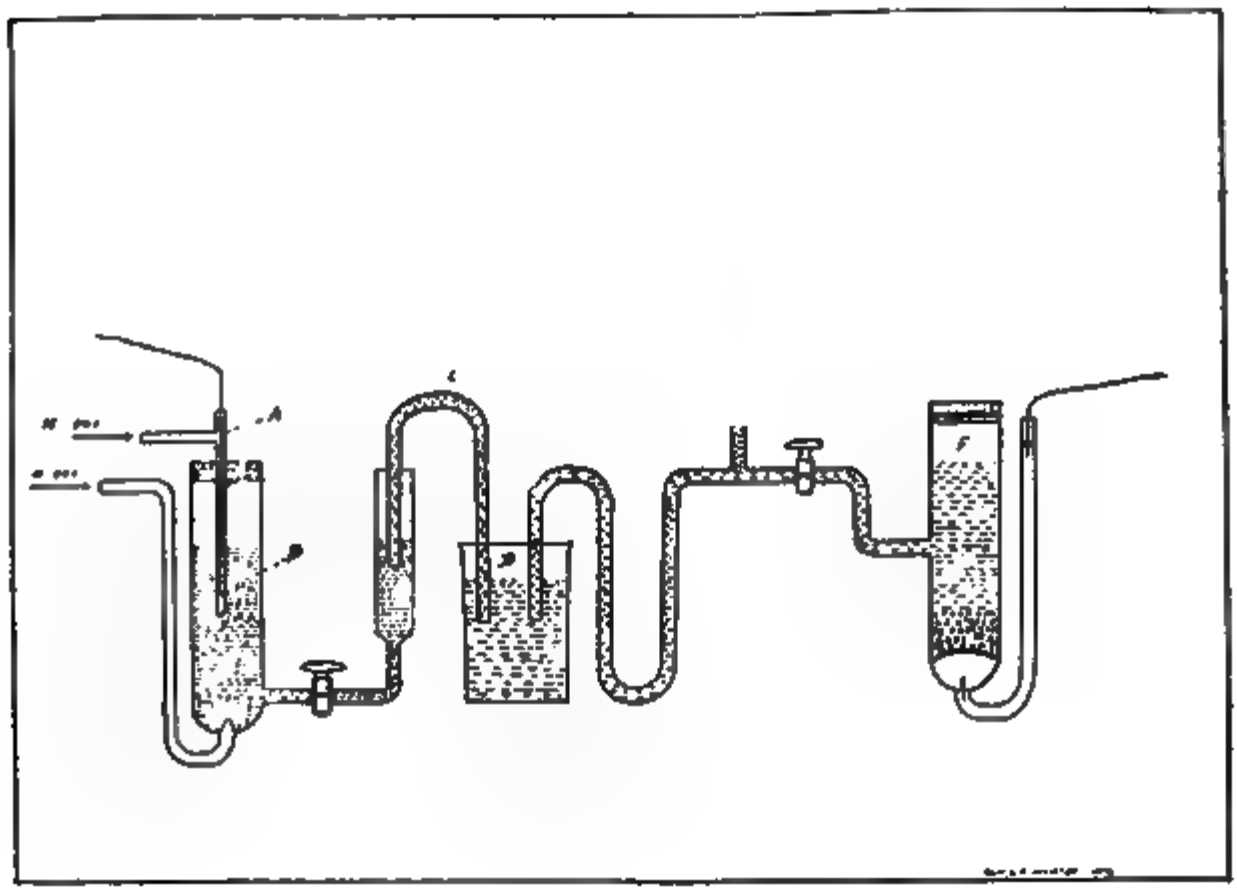

FIG. 11.—Modified Cottrell hydrogen electrode. (After Schmidt.)

In order to ascertain the actual reaction of a complex mixture of weak and strong acids and bases such as the blood or other tissue-fluids, therefore, a method of measurement must be employed which is static and not dynamic, *i.e.*, which leaves the state of the blood unaltered in respect to the balance of acids and bases which it contains. For this purpose no method is better adapted for obtaining accurate results than the electrometric or **Potentiometric Method.** The principle of this method has already been explained upon page 154. For the degree of accuracy usually required in biochemical or physiological researches the apparatus employed by Hoagland (1) and illustrated on page 272 (Fig. 10) is undoubtedly the simplest and most convenient. For solutions not containing volatile acids, the Cottrell form of electrode as modified by C. L. A. Schmidt is the best (Fig. 11), but when the fluid to be investigated contains carbon dioxide which would be

blown out by a continuous stream of gas, the electrode must be enclosed in a gas-tight vessel containing hydrogen and the fluid to be investigated and the vessel must be shaken to secure continuous contact of the electrode with hydrogen so as to maintain its saturation (Fig. 12). Certain special precautions must be taken, when potentiometrically measuring the reaction of fluids containing proteins, especially that of bringing the hydrogen electrode to equilibrium with neutral distilled water before immersing it in the protein solution, for otherwise the acid reaction of the platinum due to the great excess of hydrogen ions which it contains will precipitate many proteins in a film upon its surface. Foaming, which is often troubesome in protein solutions, may be prevented by addition of a few drops of octyl alcohol, or of a mixture of amyl alcohol and kerosine, or of isoamyl isovalerate.

FIG. 12.—"Shaking" hydrogen electrode. (After Clark.)

The potentiometric method was first employed for the estimation of the reaction of blood by Hoeber. The alkalinity of the blood which was indicated by his earliest measurements was excessive, owing to the fact that the stream of hydrogen employed to saturate the electrode blew out the carbon dioxide which in circulating blood stands in equilibrium with the bicarbonates, and contributes materially to the maintenance of neutrality. Later and more accurate measurements by Hoeber and many others are unanimous in establishing the fact that the normal reaction of the blood is so faintly alkaline as to approximate very closely to neutrality. Thus at absolute neutrality, as in neutral distilled water, the hydrogen and hydroxyl ions are equal in concentration, namely 0.8×10^{-7} normal. The actual hydroxyl concentration in the blood is only about double this, namely 1.6×10^{-7} or less than one five-millionth normal at the CO_2-pressures prevailing in the circulating blood (0.028 to 0.054 atmosphere).

Another method which has been extensively employed in the investigation of the reactions of blood-serum and of other tissue-fluids is the **Indicator-method** of Friedenthal which has been especially applied to these investigations by Sörensen. This method consists in adding to the fluid under investigation a number of different indicators known to display color-changes, at differing hydrogen or hydroxyl ion concentrations. The same indicators are also added to a series of mixtures of monosodium and disodium phosphate, of which the former is acid in reaction and the latter alkaline. The hydrogen ion concentration of all possible mixtures of these salts has been determined, and that mixture which yields most nearly the same tints with indicators as the unknown fluid evidently corresponds to it in hydrogen ion concentration. This method is not applicable to a highly colored fluid such as whole blood since the tints of indicators are not accurately appreciable in such a fluid. Furthermore it is to be noted that the indicators most suitable for this purpose are precisely those which are least desirable for the ordinary purposes of direct titration, because the best indicator for titration is that which displays a sharp change from one tint to another at a certain reaction, whereas the best indicator for the indirect method of titration just described is evidently one which offers a large number of appreciable changes of shade or tint within a limited range of hydrogen ion concentrations. The most suitable indicator for the purposes of indirect titration within the range of reactions commonly met with in tissue-fluids, is **Phenol Sulphonphthalein**. Finally it should be carefully noted that the choice of indicators is limited to those which do not react chemically with the proteins or other substances commonly present in tissue-fluids. A variety of dyes which are commonly employed as indicators in direct titration are unsuitable for our purpose because they interact with proteins and the compounds which are formed do not change color at the hydrogen ion concentration at which the free dye changes color, or even may display totally different colors from those which the free dye exhibits.

By these various methods it has been ascertained that not only is the blood of all vertebrates very nearly neutral in reaction, but almost all of the tissue-fluids are also approximately neutral. Thus the **Pancreatic Juice**, the most alkaline of body-fluids, contains 5×10^{-9} H^+, corresponding to an alkalinity of 13×10^{-7} OH^- or a little over one millionth normal. Hitherto, according to Friedenthal, no animal fluid has been found which contains less than $10^{-10} H^+$, that is, more than about $\frac{6}{100.000}$ normal OH^-.

Now the neutrality of the blood is maintained with extraordinary exactitude despite the fact that a large proportion of the products of metabolism are acid in reaction and are washed out of the tissues in which they are formed, into the blood. The products of muscular activity include carbon dioxide, lactic acid and acid phosphates, and the muscular exertion which is involved, for example, in climbing a steep hill involves the expenditure of a very considerable number of

foot-pounds of energy, and the oxidation of a correspondingly large quantity of carbohydrate material, of which the carbon is converted ultimately into carbon dioxide, which is carried to the lungs through the mediation of the blood. Yet while this large production of acid products may cause some slight distress of breathing in the unaccustomed individual, it barely perceptibly modifies the reaction of the blood. The intravenous injection of large quantities of acid produces an altogether disproportionately small effect upon the alkalinity of the blood. In **Diabetes** the faulty oxidation of fats produces a quantity of non-volatile acids which cannot be discharged as carbon dioxide is discharged, through the respiratory epithelium of the lungs, and yet in many cases of advanced diabetes the reaction of the blood is only very slightly affected so that even in diabetic coma the acidity of the blood is only raised to 1×10^{-7} normal H^+, a reaction which would be communicated to a hundred liters of neutral distilled water by the addition of a single drop of normal acid solution.

The mechanism whereby this extraordinary stability of reaction is attained is a dynamic equilibrium which involves a variety of coördinated factors. Thus the kidneys assist in removing excess of acids by excreting a predominance of acid salts and of non-volatile acids. The lungs are, however, the most important organs of acid-elimination, since they contribute to the reduction of the hydrogen ion concentration of the blood by permitting the escape of carbon dioxide. On the other hand the tissues themselves can contribute to the neutralization of injurious excess of hydrogen ions by arresting the formation of urea from protein nitrogen at the intermediate stage of ammonia, the ammonium salts of the excessive acids being excreted as such in the urine. Hence, in **Acidosis** such as that encountered in diabetes and in many toxemias, an unusual quantity of **Ammonia** appears in the urine.

The prime agent in accomplishing the regulation of the reaction of the tissues and tissue-fluids is, however, the blood itself. This may very readily be perceived by comparing the relative powers of blood and of distilled water or sodium chloride solution to neutralize acids. If two indicators be chosen which change color at differing hydrogen ion concentrations, and distilled water and blood-serum respectively be neutralized first to one, and then to the other indicator, the difference between the two titers will be extremely small in the case of distilled water and of very considerable magnitude in the case of the blood-serum. It can be shown in fact, that provided the carbon dioxide tension be maintained at the levels which prevail in circulating blood, one hundred volumes of blood of normal reaction can neutralize no less than 125 volumes of $\frac{m}{100}$ hydrochloric acid before attaining the hydrogen ion concentration of advanced acidosis, namely 1.00×10^{-7} at 38°. This would be equivalent, in a man whose circulation contains five liters of blood, to the neutralization of over six liters of $\frac{m}{100}$ or six hun-

dred cubic centimeters of $\frac{m}{10}$ acid. In the body, it must be remembered, this remarkable neutralizing-power of the blood is assisted by the added ventilation of carbon dioxide from the lungs, which occurs in consequence of the **Dyspnœa** or rapid breathing which results from a slight decrease of the alkalinity of the blood, and by the excretion of acid salts by the kidneys and by the production of ammonia from the tissues.

The origin of the neutralizing-power of the blood is threefold: in the first place the **Bicarbonates** of the blood are capable of neutralizing large quantities of acid without any great change in the hydrogen ion concentration by undergoing the reactions:

$$NaHCO_3 + HA = NaA + H_2CO_3$$
$$H_2CO_3 = H_2O + CO_2$$

Thus if the acid HA is strongly dissociated, the effect of these transformations is to replace it by the exceedingly weakly dissociated carbonic acid or by the neutral gas carbon dioxide. In a similar manner the **Phosphates** of the blood contribute to maintain neutrality by undergoing the reaction:

$$Na_2HPO_4 + HA = NaH_2PO_4 + NaA$$

whereby the strongly dissociated acid HA is replaced by the faintly acid salt, monosodium phosphate. Finally the **Protein Salts** in the blood also assist in the preservation of neutrality by entering into reactions of the type:

$$\text{Na Protein} + HA = \text{H Protein} + NaA$$

the strong acid being in this instance replaced by practically neutral uncombined protein.

Of these three agencies the bicarbonates are quantitatively much the most important. This arises from their abundance in plasma and also from the fact that the dissociation-constant of carbonic acid, or proportion of hydrogen ions to undissociated acid in the reaction of dissociation:

$$H_2CO_3 \rightleftarrows H^+ + HCO^-_3$$

is very nearly equal to the hydrogen ion concentration in distilled water at absolute neutrality (0.8×10^{-7} normal). Now L. J. Henderson has shown that the rate of change in the alkalinity or acidity of a solution of an acid when alkalies or acids are added to it is a minimum when the dissociation-constant of the acid is of this magnitude. He illustrates this principle by the following table, showing the amount of tenth normal alkali required to secure a definite but arbitrarily chosen change in alkalinity when added to equal amounts of the undermentioned acids:

Acid.	Dissociation-constant. $\times 10^{-7}$	Cubic centimeters of alkali required.
Phenol	0.0013	0.01
Boric acid	0.017	0.08
Hydrogen sulphide	0.57	1.10
Monosodium phosphate	2.0	1.00
Carbonic acid	3.0	0.72
Picolinic acid	18.0	0.10
Acetic acid	180.0	0.03

The ability of sodium bicarbonate, in equilibrium with carbonic acid, to maintain the neutrality of its solutions is strikingly illustrated by Henderson in the following way: "Suppose, for example, a solution of 100 liters containing one kilogram of sodium bicarbonate in equilibrium with an atmosphere containing one gram of carbon dioxide per liter. Let hydrochloric acid be added in successive small portions to the solution. Further, let the solution be constantly stirred and shaken, and let the experiment be conducted slowly, so that there shall always be equilibrium between the carbonic acid in the solution and in the atmosphere. Further, let the temperature be such that the absorption-coefficient of carbon dioxide shall be 1.000. Then the successive states of the solution will be approximately as recorded in the following table.

HCl added, grams.	Ratio of H_2CO_3 to $NaHCO_3$.	H^+ normal.	OH^- normal.	Relative acidity.	Relative alkalinity.
0 . .	2.27 to 11.90	0.000000057	0.000000176	0.57	1.76
10 . .	2.27 to 11.50	0.000000059	0.000000170	0.59	1.70
50 . .	2.27 to 10.00	0.000000068	0.000000147	0.68	1.47
100 . .	2.27 to 8.20	0.000000083	0.000000120	0.83	1.20
150 . .	2.27 to 6.30	0.000000108	0.000000093	1.08	0.93
200 . .	2.27 to 4.40	0.000000154	0.000000065	1.54	0.65
250 . .	2.27 to 2.60	0.00000026	0.000000039	2.60	0.39
300 . .	2.27 to 0.68	0.0000010	0.000000010	10.	0.10
310 . .	2.27 to 0.31	0.0000022	0.0000000045	22.	0.045
318 . .	∞	0.00026	0.00000000039	260.	0.0039
320 . .	.....	0.00045	0.00000000022	450.	0.0022
330 . .	.....	0.0027	0.000000000037	2700.	0.00037

"From the beginning of the experiment until almost 250 grams of hydrochloric acid have been added, neither alkalinity nor acidity is double in intensity the values which obtain in a perfectly neutral solution." "Such close approach to neutrality can be attained with pure water only after elaborate and very difficult purification, yet in the presence of carbonic acid it is the natural condition."

In laboratory-glassware a mixture of disodium and monosodium **Phosphates** would perhaps be almost as efficient as sodium bicarbonate in preserving neutrality. In the body, however, they are not so efficient as the bicarbonates because in the first place they are not nearly so abundant and in the second place the elimination of the acid phosphates which are formed in the neutralization of acids has to take place by the relatively slow and roundabout channel of the kidneys, while the elimination of carbon dioxide takes place rapidly through ventilation from the lungs.

Direct determinations by the potentiometric method have shown that the proteins contribute just about one-fifth of the neutralizing-power of the blood. In the tissues their proportional importance in maintaining neutrality is probably greater, because they are present therein in higher concentration than they are in the blood.

Solutions such as those of sodium bicarbonate, disodium phosphate or sodium proteinate which conserve the neutrality of the water in which they are dissolved are very frequently designated "**Buffer-solutions**" from the resemblance of their action obliterating rapid changes of hydrogen concentration to the action of a buffer on a vehicle in obliterating dangerously sudden changes of velocity of motion. Buffer-solutions are frequently employed now, and must necessarily be more and more widely employed, wherever stable conditions of environment are requisite, as in bacterial cultures, cultures of living tissue *in vitro*, aquarium-media for marine or fresh-water organisms, and artificial circulating media.

An estimation of the very greatest importance in all disease-conditions or metabolic disturbances which involve **Acidosis** is that of the **Alkali-reserve** or neutralizing-power of the blood. When large quantities of "fixed" *i.e.*, non-volatile acids are thrown into the blood the sodium with which they combine is rendered unavailable for neutralizing other portions of acid or for binding carbon dioxide. The alkali-reserve in such cases is diminished and the ability of the blood to maintain its neutrality is proportionately impaired. A low alkali-reserve is therefore, in general, a relatively hazardous condition.

Various methods have been proposed for measuring the alkali-reserve, the majority depending upon the fact that as the sodium bicarbonate of the blood has been diminished and the uncombined carbon dioxide stands in almost constant proportion to it, the carbon dioxide obtainable from the blood by a standard procedure is diminished. The method suggested by Van Slyke, and now employed very widely, consists in taking a sample of blood from a vein in the forearm and introducing it into a vessel filled with the alveolar air of the patient obtained by breathing and rebreathing into the vessel several times. The blood is then shaken up with the alveolar air to bring it into equilibrium with the carbon dioxide contained therein and a measured sample, without loss of carbon dioxide, is introduced into a special form of gas-burette (Fig. 13) and acidified with sulphuric acid to decompose the bicarbonates. The chamber containing the sample is now evacuated by means of a column of mercury and the gas which is evolved is measured at atmospheric temperature and pressure.

An alternative and perhaps preferable method which is, however, somewhat less simple to manipulate, consists in directly analyzing the carbon dioxide content of **Alveolar Air** obtained by rebreathing into a closed vessel. When the alkali-reserve is low, the carbon-dioxide content of the blood being diminished, the carbon-dioxide output through the lungs and the partial pressure of carbon dioxide in the alveolar air are correspondingly diminished.

Another feasible method of measuring the alkali-reserve, or, which comes to the same thing, the **Neutralizing-power** of tissue-fluids is that which has been employed by Marshall in the analysis of **Saliva**. The

FIG. 13.—Van Slyke's apparatus for the determination of carbon dioxide in blood. (After Van Slyke.)

various samples of saliva having been brought to a common reaction on the *alkaline* side of absolute neutrality (neutrality to phenolphthalein), the quantity of acid is estimated, by direct titration, which is necessary to bring the reaction of the fluid to an arbitrarily chosen reaction on the *acid* side of neutrality (neutrality to paranitrophenol). The measurement is in fact analogous to that employed by Henderson in estimating the power of different acids to maintain the neutrality of their solutions. Of course, to obtain physiologically interpretable results with blood-serum it would be necessary to carry out the titrations under a standard partial pressure of carbon dioxide, for example in a vessel filled with alveolar air. The choice of indicators is limited when the fluid under investigation is even faintly tinged with color. For example the faint yellowish tinge of blood-serum interferes with the sharpness of the end-point with paranitrophenol.

We thus see that by a variety of interlocking mechanisms, consisting in every instance of dynamic equilibria, the tissue-fluids of the higher animals, which are to their individual cells the external media in which they live, are kept extraordinarily constant in concentration, composition of mineral constituents, and hydrogen ion concentration.

The very great susceptibility of most of the chemical reactions which are involved in life-phenomena to slight changes of reaction, may very readily be seen to involve relative stability of reaction as a requisite to the orderly performance of life-processes. It is in fact an almost universal rule, in the words of Loeb, that "life-phenomena occur in a neutral liquid." The ocean which is the original home of life, is, thanks to the presence of bicarbonates and phosphates, a "buffer"-solution and nearly neutral in reaction despite the life which swarms therein. According to Palitzsch the extreme variation in the hydrogen ion concentration of the ocean is from 1.1×10^{-8} N to 0.45×10^{-8} N H^+, corresponding to an exceedingly faint alkalinity of the order of that found in the blood of mammals. In a very few instances only does life subsist in a medium which deviates far from neutrality. When secreting gastric juice, in the absence of neutralizing substances, the cells of the gastric mucosa are bathed on the side toward the lumen of the stomach by a fluid which may attain an acidity or hydrogen ion concentration due to hydrochloric acid of no less than one hundredth normal, or ten thousand times the acidity which would correspond to the alkalinity of pancreatic juice. The "salivary glands" of certain carnivorous molluscs, which probably correspond in function to the gastric glands in *mammalia*, similarly secrete an acid juice in which the high hydrogen ion concentration is attributable to **Sulphuric Acid.**

With such rare exceptions, exhibited only by highly specialized and adapted cells, the immediate environment in which living matter subsists is extremely invariable in certain physical characteristics, and this invariability which is essential to the normal occurrence of life-phenomena, is brought about through the interplay of unique

physical and chemical properties which are possessed by water and carbon dioxide. Even the additional stability of the environment of life which is brought about by the maintenance of constant temperature in the homoiothermal animals, is dependent upon the unique specific heat of water. The fitness of our environment for life is therefore essentially dependent upon these substances. As Henderson has pointed out, it is not that living matter has become adapted in an evolutionary sense to this medium, although specific organs concerned in the maintenance of the stability of the environment in higher organisms, such as the kidney, may have been subject to evolutionary adaptation. For the environment or the conditions from which it inevitably arose long antedated life itself, and the earliest forms of life must have been fitted to this environment no less exactly than the later. A direct chemical interrelationship between life phenomena and the particular type of environment in which they occur is thus indicated.

It is somewhat idle to speculate whether or not life could subsist in some quite different environment with some other element such as silicon or boron as a base instead of carbon. Such "life," if it could correctly be so called, lies of necessity outside our experience. But the absolute dependence of life as we know it upon water in the liquid form and carbon dioxide in the gaseous form, renders the temperature limits between which life can subsist excessively narrow in comparison with the vast range of temperatures found in the various portions of our universe. Of the 6500 degrees which separate the temperature of interstellar space from that of the surface of the sun, only 65° or one per cent. of the total range is suitable for the occurrence of life-phenomena. In view of this exceedingly narrow margin upon which life precariously depends, the probability of its presence in any other of the bodies in our solar system must be regarded as exceedingly small. Concerning the possibility of life in other suns or planets which may be associated with them, we are of course in complete ignorance, but Arrhenius has put forward the interesting hypothesis that life may be transmissable, in the latent form which is embodied in bacterial spores, from one part of the universe to another in association with cosmic dust. **Bacterial Spores** have been experimentally shown to be exceedingly resistant to desiccation and low temperatures, retaining their ability to give rise to functionally active protoplasm so soon as they encounter a favorable environment. The computations of Arrhenius show that the known properties of certain bacterial spores are not inconsistent with the view that they might survive a journey through space, impelled by light-pressure, from one solar system to another. If this view be correct, then the existence of life in any part of the universe might sow the whole with seeds ready to develop at any momoment at which the environment of a particular cosmic body becomes suitable for the maintenance of the processes of functionally active life.

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CHAPTER XIII.

PROPERTIES CONFERRED BY THE COLLOIDAL CONSTITUENTS: STRUCTURE AND CONSISTENCY.

THE EMULSION-STRUCTURE OF PROTOPLASM.

One of the most important aspects of the relationship of the **Lipoids** to the properties and behavior of protoplasm is that arising out of the marked effect upon the tension of protoplasmic surfaces which the lipoids and their decomposition-products are capable of bringing about. The **Surface-tension** of the interface between water and gas, or an immiscible fluid or solid, is very markedly reduced by **Oils, Fatty Acids** or **Soaps,** and this fact contributes in the first place to the determination of the distribution of these substances in the cell, and in the second place to the stability of the emulsified substances in living cells, which, despite their immiscibility in water, remain suspended in the form of stable emulsions within the material composing the protoplasm.

The distribution of soluble fatty materials in the cell, such as the **Lecithins** must be considerably influenced by the extent and variety of the surfaces which are presented by the sponge- or foam-like structure of protoplasm. The reason for this is that all those substances which reduce superficial tensions also tend, if possible, to become concentrated upon any surfaces presented to them. This is very strikingly shown, for example, in a classical experiment adduced by J. J. Thomson. If a deeply colored solution of **Potassium Permanganate** be passed through a long column of well washed and finely ground quartz-sand, the first few drops of fluid which percolate through the column will be found to be colorless, the whole of the permanganate in this first quantum having been abstracted from the solution by the surfaces over which it has passed, not because of any chemical interaction between the sand and the reagent, but in consequence of the reduction of the tension of the water-sand interface by the permanganate. Similarly, if aqueous solutions of **Saponins** or of **Bile-salts** be shaken up with petroleum-oils, the dissolved substance will be found to have become concentrated at the surface of the oil-drops, and in the foam which forms when saponin solutions are shaken in air, the saponin is more concentrated than it is in the body of the liquid.

The mechanism of this retention of dissolved substances by surfaces is as follows: In the accompanying diagram (Fig. 14) of a spherical droplet partially enclosed by a layer of molecules which coat it and separate it excepting at the gap A from direct contact with the surrounding medium, if the enveloping molecules reduce the

tension of the interface between the drop and the medium in which it is suspended, it is evident that the tension of the exposed gap in the surface will be greater than the tension of the covered portions of the surface. The two portions of the surface will be pulling unequally, therefore, and unbalanced excess of tension will exist at the gap in the sense indicated by the arrows, and the tendency of this tension in the case of a gap of molecular dimensions will obviously be to draw together the edges of the enveloping film and reduce the tension of all parts of the surface to a uniform value. Any molecules of such a substance coming into contact with the surface will therefore tend to be held or "trapped" there, and since, in the course of the fortuitous motions of the dissolved molecules, a very large number must repeatedly come into contact with any surfaces exposed to a solution, the accumulation of adhering molecules will continue until the droplet becomes covered by a layer of such thickness that the molecular attraction between the underlying molecules of the drop and those of the surrounding medium becomes inappreciable owing to the distance through which it has to be exerted. This will occur when the thickness of the film is of the order of that of a soap-bubble just before it bursts, namely about one two millionth of a millimeter.

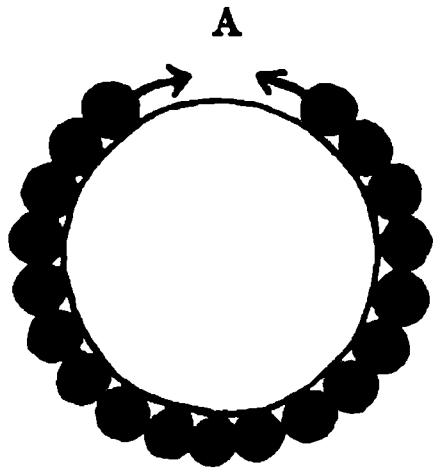

FIG. 14.—Illustrating the tendency of a lipoidal layer at the interface of two aqueous phases to repair itself when broken.

The extent to which the **Surface-tension** of water is reduced by lipoidal substances and soaps may be inferred from the following results reported by Lord Rayleigh. The measurements refer to an air-water interface.

	Dynes per linear c.m.
Tension of pure water[1]	74
Tension of greasy water	33
Tension of water saturated with olive oil	41
Tension of water saturated with sodium oleate	25

The trace of **Olive Oil** which dissolves in water, therefore, reduces the tension of the air-water interface to one-half its normal magnitude.

The lipoids in the cell are present partly in soluble forms, such as

[1] The tension of the air-water interface is usually considered to be 81 dynes per square centimeter which is the estimate of Quincke. The estimate of Lord Rayleigh who employed very refined method of measurement is more probably correct. In any case, all of the estimates having been made by the same method, they are comparable with one another.

the **Lecithins**, partly in a very finely emulsified form, the individual particles being of ultramicroscopic dimensions, and partly in a coarsely emulsified form such as that found in fatty connective tissues. The presence of a large proportion of ultramicroscopically divided fat is shown by the high fat-content of many tissues in which microscopical examination after appropriate staining fails to reveal the presence of visible fat-globules. Under certain conditions, especially in **Phosphorus-poisoning** and in **Anaphylactic Shock**, the ultramicroscopic particles in certain tissues coalesce to form coarse emulsions and the particular tissues affected, as for example the liver in phosphorus-poisoning, are then easily seen to be heavily infiltrated with fat. Direct analysis, has shown that in such cases the fat-content of the tissues is nevertheless normal, in other words the normal liver-cell contains just as much fat as the liver-cell which has undergone fatty degeneration in consequence of phosphorus-poisoning, but in the normal cell the **Emulsification** of the fat is so thorough that the greater part of the fat is present in particles too small to be visible under the microscope. The soluble lipoids and the soaps and other substances which reduce the tension of an oil-water interface are probably in large proportion concentrated at the extensive surfaces which arise from this subdivision.

The **Emulsification** of fats in water is greatly facilitated by the presence in the water of a substance which reduces the interfacial tension, provided that at the same time the substance forms a viscous or sparingly soluble coating over the oil-droplets which retards their coalescence when they come fortuitously into contact with one another. We have already had occasion to dwell upon the importance of soaps and of the bile-salts in bringing about the emulsification of the fats in the diet prior to their hydrolysis by the digestive enzymes.

When olive oil is shaken up with pure water little or no emulsification occurs. Even when the mixture has been very thoroughly shaken, the oil and water separate completely within a comparatively brief period. If, however, a little sodium carbonate or hydroxide be added to the water in order to form soap with the trace of fatty acid which oil contains, the effect of shaking the mixture is now very different. A milky or creamy emulsion is formed with comparatively little expenditure of mechanical effort in shaking, and no separation of the two fluids will occur even after long intervals of time. The emulsifying action of alkalies is also strikingly illustrated by floating drops of **Olive Oil** upon distilled water and one per cent. sodium carbonate solution respectively. In the latter case the oil-droplet spreads out, fluctuations of superficial tension at the edges of the drop cause deformations, and result in a species of "fraying" of the edges, minute particles of the oil breaking off to form a milky emulsion which gradually spreads through the solution.

In the **Emulsions** of oil in water which are thus formed the spherical droplets of oil are surrounded and completely enveloped by the water. The power of a given quantity of water to surround oil must evidently

be limited, however, for no matter how tightly packed the particles of oil may be, the thickness of the layer of water between them cannot be of less than molecular dimensions at its thinnest, and must of course be much greater in the interstices of the emulsion. In other words a limited quantity of water cannot emulsify an unlimited quantity of oil and, as a matter of fact, when a given quantity of oil is shaken with varying proportions of alkaline water, if the volume of water is below a certain critical fraction of the volume of the oil, the character of the emulsion which is obtained is altered altogether, and we now have emulsions of **Water in Oil.** A convenient method of symbolically representing these differing types of emulsions is to enclose the **Internal Phase** of the emulsion in brackets. Thus an emulsion of oil in water would be designated:

water (oil)

while an emulsion of water in oil would be distinguished by the symbol:

(water) oil

When the proportion of water to oil is in the neighborhood of the critical ratio, complex intermediate forms of emulsion may be encountered, such as emulsions of oil in water emulsified in oil, thus:

((oil) water) oil

It is, in fact, highly probable that the majority of emulsions of water in oil are really of this more complex type.

The character of an emulsion obtained by shaking together olive oil and alkaline water may very readily be ascertained without microscopical examination by the simple device of sprinkling upon the surface of the emulsion a little finely powdered **Sudan III** or **Scarlet R.** These dyes are soluble in oil, but insoluble in water. Hence if they are sprinkled upon the surface of an emulsion of oil in water, the dye simply dissolves in and stains the drops of oil with which it comes into actual contact, leaving the remainder of the emulsion unstained. If, however, the emulsion is one in which water is the internal phase and oil the external, the dye dissolves in the interstitial oil and spreads over the surface of the emulsion.

The following are illustrative results obtained by shaking together olive oil and water at an approximately uniform rate of shaking, and in the presence of a fixed proportion of alkali:

Components of emulsion.			
Oil c.c.	Water c.c.	5N.NaOH c.c.	Character of the emulsion obtained.
99	..	1	(Water) oil; fluid, yellow.
98	1	1	(Water) oil; fluid, yellow.
96	3	1	(Water) oil; fluid, yellow.
92	7	1	(Water) oil; fluid, creamy.
91	8	1	(Water) oil; fluid, creamy.
90	9	1	Water (oil); white, very viscous.
89	10	1	Water (oil); white, very viscous.

The critical ratio was in this instance:

$$\frac{\text{water}}{\text{oil}} = \frac{9.5}{90.5} = 0.105$$

The value of the critical ratio varies with different samples of oil, because of their varying fatty-acid content. It also varies with the proportion of alkali employed, since if the quantity of soap be insufficient to surround all the droplets with a layer of molecular thickness the stable emulsification of the whole of a large excess of oil becomes an impossibility, and the critical ratio is increased.

It will be observed that upon passing the critical ratio the characteristics of the emulsion change very markedly. Instead of the yellow, fluid emulsions obtained while water is the internal phase, creamy and more viscous emulsions result when water is the external phase. In the neighborhood of the critical ratio the viscosity of the water (oil) emulsions is very greatly enhanced, and emulsions of an almost butter-like consistency may be obtained. This probably arises from the fact that when the water in the emulsion is just, and only just sufficient to surround all of the oil-droplets, any deformation whatever of the tightly-packed oil-droplets must increase the size of the interstices between them; but this can only be accomplished by a complete disruption and inversion of the emulsion; since the water is already stretched to the utmost limit of its covering-power. The viscosity, or resistance to deformation of these emulsions, therefore, represents the force required to invert their structure.

Not only the lipoid constituents of cells, but also the **Proteins** tend to form films at the surfaces of suspended droplets, and thus facilitate the formation of emulsions. If **Chloroform** be shaken up with pure distilled water no emulsion arises; the two liquids separating completely after a very brief interval. If, however, a protein be added to the water the chloroform, instead of separating out in the form of a continuous layer, separates out in small discrete droplets which, if numerous, form a milky layer at the bottom of the vessel; by transmitted light, however, they appear perfectly transparent. These droplets are extraordinarily stable and do not coalesce, however long they may stand in contact with one another. They may be repeatedly washed in water until all traces of protein have disappeared from the wash-fluid, and they still remain perfectly stable and distinct from one another. They may be shaken up in chloroform itself or treated with dilute sodium hydroxide solution without impairing their form or stability. If, however, they be heated to nearly the boiling-point of chloroform under a layer of water the droplets burst and coalesce, forming a homogeneous layer of chloroform. If treated with alcohol they immediately dissolve leaving a fine membranous precipitate of protein floating in the water. Thus if we shake up chloroform with about twice its volume of a one per cent. solution of **Protamine Sulphate** or a one per cent. solution of **Gelatin**, and, after allowing the droplets to settle, pour off the supernatant fluid and repeatedly wash the droplets with water, then if we suspend these droplets in a small amount of water and add to the water an equal volume of **Alcohol** and gently shake the test-tube, the droplets which are thus stirred up into the

alcohol-water layer can be seen to swell up rapidly and burst, and the fine membranes which surrounded them can then be seen falling down through the alcohol-water. If we now add several volumes of alcohol and shake up the liquid, the chloroform droplets all disappear and what we now have is a clear, homogeneous solution, in which innumerable minute membranes can be clearly seen floating.

The phenomena of **Relative Semipermeability** may also be illustrated by these droplets. Substances which are soluble in water and also in chloroform penetrate the membranes, and if, like **Alcohol, Ether** or **Ethyl Acetate** they chance to be more soluble in chloroform than in water, the chloroform in the droplets may take up so much of the substance that they swell to the extent of rupturing their enveloping membranes. If, however, the substances in which the droplets are immersed are sufficiently insoluble in water they fail to penetrate the membranes and then the droplets may be "plasmolyzed," that is, the chloroform may be extracted from them leaving the enveloping membranes shrunken and empty. This occurs when the droplets are suspended in **Toluol, Xylol** or **Carbon Bisulphide.**

Fat emulsions which contain protein tend to form films at surfaces with which they come in contact, consisting of a more concentrated emulsion, both in respect to fat and in respect to protein, than that which constitutes the body of the liquid. This is very well illustrated by the film which forms on the surface of **Milk** when it is heated. The heating of the milk renders the **Calcium Caseinate** which it contains somewhat less soluble, and the concentrated layer of calcium caseinate and fat particles which forms at the surface becomes, at temperatures above 45°, sufficiently viscous to assume the consistency of a semi-solid film, which, owing to its high viscosity, does not readily pass back into solution upon cooling. A pure solution of calcium caseinate becomes markedly opalescent on heating to 45° but does not form a sufficiently viscous film at its surface to be mechanically separable from the underlying liquid.

A living cell consists essentially of a more or less finely emulsified suspension of fat-like substances in a semi-gelatinous solution of protein. The film which forms at the surface of warm milk may be regarded as an extreme illustration of the type of surface-layer which we may therefore expect to exist at the periphery of living cells, namely an emulsion of fat and protein, more concentrated and, therefore, more viscous than the emulsion which constitutes the underlying protoplasm.

The emulsion-structure of the superficial layer in cells enables us to account for a very widespread property of living cells which would otherwise be almost inexplicable, namely the property of **One-sided Permeability.** This phenomenon is very well illustrated by the following experiment of Overton's: If tadpoles are immersed in a five or six per cent. solution of cane-sugar or a 0.6 per cent. solution of sodium chloride they are unaffected either in size or in any other notable

respect. If, however, they are immersed in solutions which are *hypertonic* to these, for example in eight per cent. cane-sugar or 0.8 per cent. sodium chloride, they lose a quantity of water and in twenty-four hours they are found to have shrunk decidedly in volume. Evidently, then, the sugar or salt cannot enter the limiting membranes of the cells of the skin while water can pass through them freely in the direction tissues → external medium. One might imagine, therefore, that the epithelium of a tadpole resembles an ordinary semipermeable membrane, permitting the passage of water but not of dissolved substances. If this were really the case, then on immersing the tadpoles in solutions which are *hypotonic* to 0.6 sodium chloride we should expect them to take up water and to increase in volume just as much as they decrease in volume in hypertonic solutions. This does not occur, however, and tadpoles immersed in hypotonic solutions do not take up water to any greater extent than from isotonic solutions. We can only infer, therefore, that the superficial epithelium of the tadpole permits the passage of water from within outward, but not in the reverse direction; that this membrane is permeable to water in the direction tissues → external medium, but not in the direction external medium → tissues.

The property of one-sided permeability is displayed by many living membranes, but not by all. A very striking contrast is shown in this respect by the **Pavement Epithelium** which lines the peritoneal cavity, on the one hand, and the **Columnar Epithelium** which lines the lumen of the small intestine, on the other. Thus Heidenhain introduced 50 c.c. of a three per cent. solution of **Glucose** into the peritoneal cavity of a dog, and at the same time 44 c.c. of the same solution into an isolated loop of the intestine. After ninety minutes the quantity and composition of the residual fluid in the peritoneal cavity were as follows:

Quantity of fluid.	Glucose.	Sodium chloride.
19.5 c.c.	1.0 per cent.	0.55 per cent.

while in twenty-five minutes the composition of the residual fluid in the loop of intestine was as follows:

Quantity of fluid.	Glucose.	Sodium chloride.
19.0 c.c.	3.8 per cent.	0.04 per cent.

From the peritoneal cavity both water and glucose had issued into the tissue-fluids, the glucose even more rapidly than the water, while sodium chloride, which was absent from the fluid originally introduced, had diffused from the tissues into the peritoneal cavity. The peritoneal epithelium, therefore, behaved like a membrane of parchment, permitting the passage of dissolved substances in either direction in proportion to their relative concentrations on the two sides of the membrane.

From the intestinal loop, both water and glucose had issued into the tissue-fluids, water somewhat more rapidly than glucose. But practically no sodium chloride had diffused into the intestinal fluid from the

tissue-fluids. Evidently the intestinal epithelium permits the passage of certain dissolved substances into the tissue-fluids behind it, but not the migration of dissolved substances in the reverse direction.

The maintenance of one-sided permeability in tissues is dependent upon the maintenance of the unimpaired structure of the cells. Thus the phenomenon of one-sided permeability is nowhere more clearly illustrated than it is in the kidneys, where the dissolved constituents of **Urine** are constantly excreted against a high pressure, the tissues of the kidney being much more permeable for dissolved substances in the direction blood → urine than in the direction urine → blood. If, however, the epithelium of the renal tubules is injured by perfusion with solutions of certain substances, for example **Sodium Fluoride**, it loses this power and comes to resemble much more closely a membrane of parchment. This is very clearly illustrated by the following experiment by Bottazzi. One kidney in a dog was injured by perfusion with sodium fluoride solution. The ureters of the two kidneys were then separately catheterized and the freezing-points of the samples of urine collected from the two kidneys were determined from time to time. The following were illustrative results:

Time.	Urine c.c.:		Depression of freezing-point.		Depression of freezing-point of blood.
	Normal.	Injured.	Normal.	Injured.	
2.30 to 3.00	9.	12	1.616	0.979	0.572
3.30 to 4.00	14.	20	1.118	0.294	
4.50 to 5.20	14.	22	0.584	0.240	
5.30 to 6.00	10.	22	0.570	0.224	
6.00 to 6.30	12.	20	0.572	0.212	0.560
9.30 to 10.00	4.	9	1.002	0.206	
8.00 to 8.30	2.5	6	1.304	0.302	0.569

It is evident that the phenomenon of one-sided permeability must be dependent upon a heterogeneous structure of the membrane which displays it. The phenomenon is not, and could not be displayed by structureless membranes, or by membranes having a uniform structure in the direction of penetration, *i. e.*, perpendicularly to their surface. For instance, consider a membrane formed of successive columns, of two different materials, one of which permits the passage of substances soluble in water while the other does not. Then if the arrangement of these two components were that displayed in Fig. 15 substances soluble in water could penetrate the unshaded channels just as easily from below as from above the membrane. But if the membrane were curved, so as to bring the columns of impenetrable material closer together on the under than on the upper surface, or if they were pyramidal in shape so as to achieve the same end, so that the arrangement would be that displayed in Fig. 16 then it is evident that the penetrable area on the under surface of the membrane would be a much smaller proportion of the whole area than on the upper surface of the membrane, so that substances penetrating from above would do so with comparative ease, while substances issuing from below the membrane would do so with difficulty.

Now the lipoid constituents of the cell may be assumed to be generally impenetrable by substances which are insoluble in fats and oils, so that these must seek entry to the cell through the interstitial spaces between the lipoid constituents of the superficial membrane of the cell. If, therefore, these intersititial spaces filled with a solution or gel of protein, were so constructed as to be narrower at one end than at the other, the superficial membrane of the cell would evidently be more readily permeable in one direction than in the opposite.

The most usual spatial arrangement of the various structures or constituents of a cell is that of **Radial Symmetry**. The primitive arrangement of strictly radial symmetry so frequently displayed in spherical cells becomes modified or distorted in those cells, such as the majority of epithelial cells, which, through mutual compression or for other reasons, have assumed a columnar, stratified or flattened outline. In such cases the radial arrangement of structures may be confined to the sides or margins of the cell, and differ in character in the protoplasm underlying the various facets of the cell.

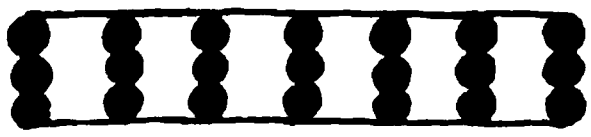

FIG. 15

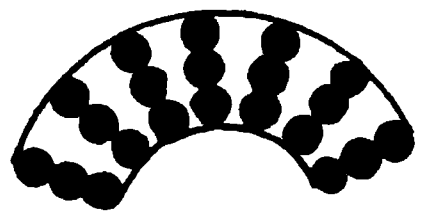

FIG. 16

FIGS. 15 and 16.—These figures illustrate the effect of curvature of the surface of a radially dispersed emulsion in producing funnel-shaped interstitial pores between the radiating columns of lipoid globules. In Fig. 15 both surfaces of the cortical layer being plane, the diameter of the interstitial orifices are the same at the exterior and interior surfaces. In Fig. 16 the cortical layer being curved, the interstitial orifices are narrower upon the under than upon the upper surface of the cortical layer.

A radial arrangement of the ultramicroscopic fat-granules of the cell would obviously lead to the formation upon the surface and in the subjacent protoplasm of minute **Funnel-shaped Pores**, of which the interstitial openings would be permeable to substances soluble in water, while the walls, being composed of fat-granules, would be impermeable or with difficulty permeable by such substances. The interstitial openings at the margin distal from the center, from which the fat-granules radiate, would be relatively large, while at points lying nearer to the center of radiation, that is, in general, deeper within the cell, the diameter of the pores would be very considerably contracted.

Substances which are soluble in water might evidently penetrate such a cell with relative ease, since a relatively large proportion of the exterior cell-surface would consist of the water-soluble phase of the emulsion, but they would issue from the interior of the cell with relative difficulty, since a relatively large proportion of the area which they would have to traverse to find an outlet would consist of the lipoidal phase. If the modifications of radial symmetry which are so characteristic of **Epithelial Cells** should result in the confinement of this structure to one surface or facet of the cell, it is obviously possible that one-sided permeability of a tissue composed of such cells might

be the consequence. This may be seen by reference to the diagram in Fig. 16.

It should be borne in mind that the existence of funnel-shaped pores in the surface of a cell or in a membrane would only give rise to one-sided permeability provided the diameter of the pore at the constricted end were comparable with the mean free path of the penetrating molecules. Were the least diameter of the pores *less* than the mean free path of the pentrating molecule, then the membrane would be a strictly semipermeable membrane for this type of molecule. Were the least diameter of the pores on the contrary, very large in comparison with the mean free path of the molecule concerned, then the membrane would be freely permeable by this molecule in either direction. Thus it is readily conceivable that membranes of this type might exhibit **One-sided Permeability** for certain substances dissolved in water, **Absolute Permeability** for others, and **Semipermeability** for yet other molecules.

On the other hand, if the above sketch represents truly the structure of the superficial layer of cells, substances which are soluble in fats would enter the cell through the radiating columns of lipoidal material. Now the phenomenon of one-sided permeability has not as yet been observed to be displayed toward substances which are soluble in lipoids. Indeed, in general, the penetrability of cells by substances which are soluble in lipoids is very much greater than their penetrability by other substances, no matter how soluble they may be in water. This fact is very strikingly illustrated by the experiments of Overton and Meyer, who measured the minimal concentrations of various **Narcotics** dissolved in water which would induce narcosis in tadpoles, the narcosis being evidenced by cessation of movement. The same narcotics were dissolved in water and the water shaken up in **Olive Oil** and the relative solubilities of the narcotics in water and in oil estimated by the distribution of the narcotic between the two solvents. The following were illustrative results obtained with various **Alcohols**:

Narcotic.	Critical narcotizing concentration in gram-molecules per liter.	$\frac{\text{Solubility in water.}}{\text{Solubility in oil.}}$
Methyl alcohol	0.52–0.62	Solubility in water = ∝ soluble in 50 parts of oil.
Ethyl alcohol	0.27–0.31	30 : 1
Propyl alcohol	0.11	8 : 1
Butyl alcohol	0.038	Soluble in 12 parts of water; solubility in oil = ∝.
Caprylic alcohol	0.0004	Soluble in 2000 parts of water; solubility in oil = ∝.

The following results were obtained with other narcotics:

Narcotic.	Critical concentration.		$\frac{\text{Solubility in oil.}}{\text{Solubility in water.}}$	
	At 3°.	At 30°.	At 3°.	At 30°.
Salicylamide	1/1300	1/600	22.232	14.002
Benzamide	1/500	1/200	0.672	0.437
Monacetin	1/90	1/70	0.099	0.066
Ethyl alcohol	1/8	1/7	0.026	0.047
Chloral hydrate	1/50	1/250	0.053	0.236
Acetone	1/8	1/7	0.146	0.235

Hence if tadpoles, anesthetized at 30° by a $\frac{m}{250}$ solution of **Chloral Hydrate** be cooled they recover their mobility, on warming they are again anesthetized, and at 30° the solubility of chloral hydrate in olive oil is much greater than it is at lower temperatures.

While the inference drawn by Overton and Meyer from these experiments, that only those substances which are soluble in **Lipoids** can penetrate the cell, obviously cannot be substantiated, for otherwise neither water, inorganic salts nor amino-acids could ever gain entry into protoplasm, yet it is very manifest from these and many other experiments of a like nature that substances which are soluble in lipoids do enter living cells with exceptional ease. We may probably infer with safety that the lipoidal elements in the superficial membranes of cells constitute a large proportion of the surface, and the interstices a relatively small proportion, so that substances which are insoluble in lipoids enter living cells with comparative difficulty

Fig. 17.—Illustrating the increase in the diameter of the interstitial spaces which results from increase in the diameter of the fat-droplets in an emulsion.

Any reagent or condition which affects the **State of Aggregation** of the fat-globules in the limiting membrane of cells must necessarily affect the diameter of the interstices between them. In general those conditions involving the formation of large aggregates would increase the permeability of tissue by enlarging the diameter of the radiating fat-droplets and, therefore, that of the interstitial spaces (Fig. 17). This is very strikingly illustrated by the eggs of certain marine forms such as the sea-urchin which, when exposed to the action of fat-solvents become permeable to water which they take up from the surrounding sea-water. The water thus absorbed accumulates in a layer just under the superficial membrane of the cell, lifting it off the underlying protoplasm and forming the "**Fertilization Membrane**" which is normally the effect of a cytolytic agent carried into the egg-cell by the head of the spermatozoön. The permeability of the surface of the cell is also increased for inorganic salts, for McClendon has shown that the **Electrical Conductivity** of a suspension of sea-urchin eggs is increased by fertilization while Osterhout has shown that an increase in the electrical conductivity of living tissues is indicative of increased permeability of the surface of the cell for inorganic salts.

Since the lipoidal droplets in cells are suspended in a gelatinous or

semi-liquid solution of **Protein** we may also assume that any condition tending to alter the consistency of the interstitial protein solution would deform the structure of the emulsion. **Coagulating Agents** especially might be expected to reduce the interstitial gel to discrete granules or separate flocculi, thus removing the obstacle to coalescence of the fat globules and the consequent coarsening of structure and widening of interstices. Corresponding to this conception we find that simple heating of a piece of frog's skin renders it freely permeable to water in either direction, instead of only in one. The effect of coagulating agents upon permeability may also be strikingly illustrated in the following way: If *paramæcia* be washed free from culture medium with pure distilled water and suspended in a solution of **Methyl Green** (free from methyl violet), the protoplasm of the infusorians takes on a faint greenish tinge, but the large pseudo-nucleus remains white and unstained. After removing the excess of methyl green by washing the organisms in distilled water, a little **Cupric Chloride** may now be added to the water. Immediately the nucleus becomes stained a deep green, indicating that the impenetrability of the nuclear membrane for the dye prevents it from being stained in the normal cell, but after the action of this protein coagulant, which kills the organisms, the permeability of the nuclear membrane is so enhanced that the dye can readily enter and even attain a greater concentration therein than it does in the cytoplasm.

THE VISCOSITY OF PROTOPLASM.

The major part of the high degree of **Viscosity** which protoplasm displays is attributable to the **Protein** which it contains. The viscosity of a protein solution increases very rapidly indeed with its concentration, so rapidly, in fact, that earlier observers were inclined to the belief that the viscosity changed suddenly at definite critical concentrations instead of changing evenly and with regularity as it does in solutions of crystalloids. Later observations have shown us, however, that the viscosity of protein solutions increases with the concentration in accordance with the usual formula $\frac{\eta}{\eta^0} = A^n$, where η is the viscosity of the solution, η_0 that of the solvent, n the concentration of the solution and A a constant, the numerical value of which depends upon the nature of the dissolved substance, and upon the temperature. The following are results obtained by Sackur, employing **Sodium Caseinate**:

n (in equivalents of sodium).	$\frac{\eta}{\eta_0}$ (15° C.).	$\log_{10}$ A.
0.01830	1.870	14.8
0.01370	1.581	14.5
0.00915	1.363	14.3
0.00547	1.202	14.6
0.00458	1.165	14.5

A remarkable feature of these results is the extraordinarily high value of A, involving a very rapid increase of viscosity with increasing concentration. For the majority of crystalloids the value of A is not greatly in excess of unity, while for sodium caseinate it is of the order of 10^{14}. This fact alone would lead us to suspect that the mechanism which produces the viscosity of these solutions is different in nature from that which produces the viscosity of solutions of crystalloids. The viscosity of a protein solution is also very greatly increased by **Ionization**, the viscosity of protein solutions being at a minimum when ionic protein is absent, *i. e.*, when the protein is uncombined with acids or bases.

Indeed a very little consideration suffices to show that the viscosity of a protein solution is of a very different type from the viscosity, for example, of solutions of **Sugar** or **Glycerol** in water. Apart from the extraordinary magnitude of A, the type of viscosity exhibited by solutions of proteins differs from the viscosity of a glycerol-water mixture in that it affords no hindrance, or very slight hindrance, to the motion of ions and of crystalloidal molecules. The velocities with which various crystalloids diffuse through **Gelatin** jellies are remarkably close to the diffusion-velocities of the same substances in distilled water. The jelly causes a very slight retardation of diffusion but the hindrance to molecular movement is disproportionately small in comparison with the enormous viscosity of the jellies.

It has repeatedly been shown that the specific mobilities of the majority of inorganic ions is the same in gelatin or agar jellies as it is in distilled water. In fact, if allowance be made for the diminution of the cross-section of the conducting field which is occasioned by the presence of gelatin molecules we find that the electrical conductivities of inorganic salt solutions in gelatin jellies are only very slightly less than those of equally concentrated solutions in pure water, implying that the ions of the electrolyte move as freely in the insterstices between the protein molecules as they would move in distilled water. This is true even when the ions are protein ions, for the dependence of the **Electrical Conductivity** of protein solutions upon their dilution is of a perfectly normal character, resembling the dependence of the conductivity of a solution of a crystalloid upon dilution, although, in the range of concentrations employed, the viscosity of the protein solution increases with its concentration very greatly, while that of a salt solution, for example, increases almost imperceptibly.

On the other hand the intimate dependence of the conductivities of solutions of electrolytes upon the ordinary types of viscosity has been commented upon, and quantitatively estimated by a host of observers. Viscosities, very much less than those of the most dilute **Jellies**, if caused by such substances as sugar or glycerol, profoundly diminish the conductive power of electrolytes. Not only inorganic, but also protein ions are very greatly hindered in their mobilities by the type of viscousness which alcohol-water or glycerol-water mixtures

exhibit. In fact, whereas doubling the viscosity of a solution of **Sodium Caseinate** by the addition of protein does not measurably affect its conductivity, doubling its viscosity by the addition of forty per cent. of alcohol reduces the mobility of the caseinate ions to one-half, and the conductivity of the solution to a still smaller proportion. In estimating the influence of viscosity upon the mobilities of protein ions we can entirely disregard that portion of the viscosity of the solution which, although comparable in magnitude with the viscosity of the solvent, is attributable to the protein itself.

There are thus two kinds of viscosity which may be displayed by solutions, the one which impedes the motion of molecules or ions, and the other which does not hinder the motion of such small particles, although it does very greatly impede the passage of the fluid through a narrow tube or the rate of oscillation of a rotating disc suspended within the fluid. The former type of viscosity is displayed by solutions of inorganic substances and the simpler organic substances, the latter type of viscosity by solutions of the proteins.

The customary method of measuring viscosity, such as the measurement of the time taken by a given volume of fluid to pass, under the force of gravity, through a specified length of a narrow tube, all involve *deformation* of the fluid, whereas the estimation of viscosity which depends upon the diffusion of molecules or ions through it, does not require any displacement of the particles of the solvent in which the diffusion is occurring. Deformation is especially resisted by protein solutions, but internal molecular motions are not impeded. This fact strongly suggests the existence of a **Structure** within solutions of the proteins. It appears very probable that the molecules of protein in solution are loosely connected with one another so as to form a meshwork or three-dimensional net throughout the body of the solution. Such a net, which, in two-dimensional section, we may picture as something analogous to a tennis-net with microscopic or ultramicroscopic meshes, would offer no hindrance to the passage through it of a quickly-moving body which is much smaller than its meshes, but to any force involving deformation of its structure, for instance to a force tending to drag it through a small tube, it would offer a very considerable resistance. In measuring the resistance which a protein solution offers to passage through a capillary tube, we are not measuring true viscosity or internal friction between adjacent molecules, therefore, but the resistance of the structure of the solution to deformation.

A common method of measuring the viscosity of fluid consists in suspending a disc within the fluid and causing it to oscillate, the decrease of the rate of oscillation being a measure of the viscosity.

When this method is applied to protein solutions, however, it is found that the decrease in the rate of oscillation of the disc is abnormally rapid, but if the liquid be slightly shaken or the disc taken out and replaced, the decrease in the rate of oscillation becomes normal again for a brief period. Evidently the protein network adheres to the disc,

so that, in the course of time, the motion of the disc is not merely opposed by the friction of immediately adjacent molecules, but by the inertia of all of the protein molecules of the fluid which are attached indirectly, through a continuous meshwork, to the oscillating disc.

JELLIES AND GELATINIZATION.

The structure which confers upon protein solutions their peculiar type of **Viscosity** leads in many cases when the solutions are sufficiently concentrated to their acquiring certain of the properties of solids. Such solutions are what we term **Jellies**, and they resemble solids in presenting pronounced resistance to deformation which, however, yields to the slightest force if its action be sufficiently prolonged. Where forces of an instantaneous character are concerned, therefore, the jellies are solids, but where forces of prolonged action are concerned they are fluids. The distinction between a solid and a jelly is, in fact, largely a matter of degree. A solid will flow under a sufficiently great pressure applied for a relatively brief period of time, but a sharp impact affects it as it affects an elastic solid, causing oscillation and recoil, but not deformation. Intermediate states of matter are afforded by such materials as **Sealing-wax** which, even at ordinary temperatures, will flow under slight pressure applied for very prolonged periods, but which under even considerable forces acting sufficiently suddenly exhibits all the brittleness of a solid.

Under certain conditions, when the meshes are sufficiently coarse, various jellies or "gels" clearly display a network or spongy structure. If an insoluble gel, such as **White of Egg** coagulated by fixatives, the gel of **Colloidion** produced by the action of chloroform upon an ether solution, common black **India-rubber**, or the hydrogel of **Silica** be examined under high magnification they can all be seen to possess a fine sponge-like structure. When, for example, a thirteen per cent. solution of egg-white is fixed with sublimate, sections are found to show a sponge-structure, or, what corresponds to a sponge in two dimensions, a network-structure. W. B. Hardy, who has especially investigated this gel, failed to obtain with acid or basic dyes any staining of the substance within the meshes of the net, and pressure applied to the gel resulted in the squeezing of fluid out of its interstices. The structure of the gel is, therefore, that of an open sponge-work of solid, containing fluid within its meshes. Direct experimentation with **Agar** jellies has shown that in a gel containing one per cent. of agar, the solid framework is a solution of water in agar, while the fluid in the interstices is a dilute solution of agar in water. Upon heating the solution the two components become miscible in each other and we obtain what appears to be a homogeneous solution. Upon the basis of these facts Hardy draws a far-reaching analogy between the jellies which liquefy when heated, and gel when cooled, and the system **Phenol-water**, which, if it contains more than 71 per cent. or less than

76 per cent. of phenol, separates, at temperatures below 80° C., into two phases, the one a solution of phenol in water, the other a solution of water in phenol. According to the view developed by Hardy the two cases differ only in the fact that upon separation of the two phases in the agar-water system the system retains a structure, while in the phenol-water system no structure is retained and the components separate into two clearly demarcated layers. Essentially, the difference between the two systems consists in this: that when the phenol-water system separates into two phases, the phases become separated by the minimal possible surface, namely a plane; while when the agar-water system separates into two phases they remain in contact over an area far larger than the minimum. In the latter case it would seem that the surface-tension at the interface of the two phases is very low, so that the force leading to the diminution of surface is small. The resistance to the diminution of the interface is also very large because of the high viscosity of the gel.

The manner in which the structure of a gel is built up can be readily observed in the ternary mixture, alcohol, gelatin and water. If 13.5 grams of **Gelatin** are mixed with 50 c.c. of water and 50 c.c. of absolute alcohol, a mixture is formed which is optically homogeneous at 17° to 20° C., but which separates into two phases at temperatures below this. Hardy thus describes the sequence of events on cooling this mixture below the temperature of gelation: "As the temperature falls below the limit a clouding occurs which I find to be due to the appearance of fluid droplets which gradually increase in size until they measure 3 $\mu.\mu$. On cooling further, these fluid droplets become solid and they begin to adhere to one another. The framework is therefore an open structure which holds the fluid phase in its interstices." "When once formed the phases have considerable stability. If the droplets are composed of a solid solution one may, by the addition of water, cause them to increase to relatively vast dimensions without their being destroyed; as they increase in size their refractive index approximates more and more to that of the external phase until they are finally lost sight of. The addition of alcohol, however, once more brings them into view and causes them to shrink. Owing to this stability, once a configuration has been established, one has to far overstep the conditions of its formation in order to destroy it. This would account for the remarkable hysteresis observed in reversible gels. . . . When water is added to a ternary mixture so as to considerably swell the droplets, the system is unstable, and the two phases mix at once when it is mechanically agitated."

In jellies of this type which are dilute with respect to the colloid constituent, therefore, the structure is that of an open sponge-work, the meshes being filled with water or a water-rich solution of the substance forming the gel while the framework of the sponge consists of anastomosing threads composed of linearly arranged globules of the water-poor phase. In such gels, therefore, the surface of the water-

rich phase is concave; in other words the water-proof phase is the **Internal Phase** of the gel, and the water-rich material constitutes the **External Phase** of the gel. If, however, to a ternary mixture of gelatin, alcohol and water which forms such a gel as that described above, more gelatin be added, the character of the gel changes entirely and its structure becomes inverted. The water-poor phase becomes concave and the water-rich phase, instead of being, as formerly, concave, becomes convex to it. On cooling such a mixture to a temperature below that at which it forms an optically homogeneous solution, droplets separate out which are poor in gelatin, while the interstitial portion of the system, which is rich in gelatin, solidifies. Thus the gel comes to possess a **Honeycomb-structure** the droplets being poor in gelatin and rich in water. This is very clearly shown by the following analyses made by Hardy:

TEMPERATURE OF THE MIXTURE, 15° C. (EQUAL PARTS OF WATER AND ALCOHOL).

Per cent. gelatin in mixture.	Per cent. gelatin in droplets (internal phase).	Per cent. gelatin in interstices (external phase).
6.7	17.0	2.0
13.5	18.0	5.5
36.5	8.5	40.0

From these analyses it is also clear that the two phases in a protein gel are not of constant composition, but may, under different conditions of total concentration, etc., vary widely in their relative and absolute gelatin and water-content. This system differs, therefore, from the system phenol-water, not only in the extent of the surface which separates the phase, but also in the variability of the composition of its phases, in this respect resembling rather the system hydrated silica-water.

The reason for this inversion of structure which occurs in concentrated gelatin **Jellies** is the same as that which is the origin of the inversion of structure in olive-oil-water **Emulsions** when the proportion of oil to water is increased beyond a certain limit. The spreading- or covering-power of water is not unlimited and therefore the amount of oil or gelatin which it can surround is correspondingly restricted.

The question has been raised whether the jelly which is formed by gelatin dissolved in water (instead of alcohol-water mixtures) really possesses a structure analogous to that observed by Hardy in ternary systems. It has been urged that this structure is an artefact arising out of partial **Coagulation** of the protein, since it is not directly visible in binary systems. The action of coagulants such as alcohol or sublimate upon jellies which already possess a structure of this type, however, is not to otherwise alter, but merely to coarsen their structure. This is due to loss of water on the part of the colloid-rich droplets with a consequent diminution of the volume of the colloid-rich phase and an increase in the volume of the more fluid interstices. This can be shown, not only

by direct observation, but also by the relative ease with which water can be expressed from the jelly before and after "fixation." From **Poiseuilles' Law** for the outflow of liquid from capillary tubes, it follows that the pressure required to express the fluid must vary approximately as the inverse fourth power of the diameter of the meshes, although, of course, the variable viscosity of the expressed fluid will be a factor introducing departures from this simple law. Now a hydrogel containing 13 per cent. of **Gelatin** at a temperature of 15° C. will endure a pressure of 400 pounds to the square inch without expression of water; after fixation with formalin or corrosive sublimate, however, the fluid can be expressed from the gel like water from a sponge, with simple hand-pressure.

Since more complete coagulation does not alter the *type* of structure possessed by jellies of partially coagulated protein, but merely coarsens it, it is a fair inference that jellies which have undergone no measure of coagulation also possess the type of structure outlined by Hardy, but that owing to its fineness the details of this structure are not visible.

The existence of a structure in jellies formed by the solution of gelatin in water is also objectively demonstrated by the observation of Liesegang, that when silver nitrate diffuses into gelatin which is impregnated with potassium bichromate, the precipitation of insoluble **Silver Bichromate** does not occur indifferently in all parts of the area of diffusion, but in concentric circles. It has also been shown by Rohonyi that when thin films of gelatin are frozen the ice-crystals are formed in concentric rings. It is difficult to clearly conceive any mechanism which would permit this in a perfectly homogeneous medium. The theory that crystallization is inhibited by the gelatin until a certain degree of supersaturation is attained might account for failure of precipitation or crystallization at certain points, but, provided the jelly were strictly homogeneous and structureless, it fails to account for its appearance at other points.

The experiments of Hardy show that on adding water to the system alcohol-water-gelatin the gelatin-rich phase progressively imbibes water until it passes by a series of insensible transitions into a **Solution** of gelatin. We have seen that solutions of protein show evidence, in the peculiar type of resistance to deformation which they display, of possessing a structure which is most easily conceived as a spongework of protein molecules with intercommunicating meshes filled with water. The **Structure** of the solution is therefore that of an attenuated jelly and there is no distinction of kind, but only of degree, between a protein solution and a protein jelly. As a matter of fact, if the **Viscosity** of a solution of gelatin sufficiently concentrated to gelatinize at room-temperature be measured at intervals while it is cooling, no sharp change of viscosity is found to occur at gelation, the viscosity of the solution just prior to that point being so great as to afford clear indication of the forthcoming semi-solidification.

The structure of protoplasm, therefore, consisting as it does of an

emulsion of lipoids suspended in a solution or jelly of protein must be very complex. Essentially it is an emulsion enclosed within another emulsion and many diversities of structure and arrangement may evidently exist. One would anticipate that the architecture of such a complex system would be profoundly affected by any factors affecting the solubility of the proteins, and therefore their affinity for water. A relative alteration of volume of the water-poor and water-rich phases of the protein emulsion must necessarily disturb all the space-relations of the enclosed fat-emulsion, and these displacements acting at the **Surface** of the cell would be equivalent to opening or shutting so many doors for the entry of water-soluble substances into the cell. The striking effects of various inorganic substances upon the **Permeability** of cells upon which we shall dwell in the succeeding chapter, probably originate in changes of the affinity of the cell-proteins for water with consequent dilatations or contraction of the constituent phases of the protein jelly and enlargement or constriction of the interstitial spaces between the lipoidal elements of the superficies of the cell.

THE OSMOTIC PRESSURE OF PROTEIN SOLUTIONS.

It was formerly believed that proteins in solution exerted, in common with other colloids, either no osmotic pressure at all, or a pressure of immeasurably small extent. More recent investigations have shown, however, that the difference in this as in other respects between the colloids and the "typical" crystalloids is merely a quantitative difference which is directly attributable to and deducible from the relatively enormous size of their molecules. Thus a one per cent. solution of **Glucose** contains $\frac{1}{18}$ gram-molecules of glucose per liter and exerts an osmotic pressure of nearly one and a quarter atmospheres, but a one per cent. solution of **Hemoglobin,** which has a molecular weight of sixteen thousand, only contains $\frac{1}{1600}$ gram-molecules of protein per liter and, therefore, may be expected only to exert an osmotic pressure of 0.014 of an atmosphere.

The direct determination of the osmotic pressure of protein solutions is a task fraught with immense difficulties, on account of the difficulty of preparing ideally pure proteins. The investigations of Graham, the originator of the distinction between crystalloids and colloids, appeared to indicate that colloids in general exert a high osmotic pressure. Subsequent investigators, however, attributed these results to an admixture of crystalloids, which, as the above numerical comparison shows, might be expected to exert a disproportionate effect upon the pressures exhibited. Starling endeavored to measure directly the osmotic pressure of the proteins in blood-serum by using for his **Osmometer** a membrane permeable to salts but impermeable to proteins, and this method has, since then, been employed in all accurate work upon the subject, since, as Reid has pointed out, it is the only method of procedure which is applicable to the problem. Such a membrane is to the colloids what an ideally semipermeable membrane is to all

dissolved substances, inclusive of the colloids. We have no assurance that any given protein preparation is totally free from impurities which may influence the direct measurement of osmotic pressure; it is, therefore, essential to employ a membrane which is permeable to such impurities and thus, if time be allowed for the system to come to equilibrium, differentiates between protein and non-protein constituents of the solution under investigation. For this purpose Reid employs a membrane of vegetable parchment, which, as he has shown, is permeable even to nucleic acid, although it is impermeable to the proteins which he employed in his investigations. By extremely prolonged purification Reid has succeeded in obtaining preparations of **Egg-albumin** which exhibit no measurable osmotic pressure when examined by this method. In subsequent investigations, however, he obtained osmotic pressures, due to dissolved **Hemoglobin** of perfectly constant value and such as to indicate a molecular weight of about 48,000. Barcroft and Hill have, however, demonstrated by thermodynamical methods that in solutions containing hemoglobin prepared by less prolonged dialysis the molecular weight of this substance is close to 16,669 which is the figure calculated from the content of **Iron**, assuming each molecule of hemoglobin to contain only one atom of iron. Roaf, employing the differential osmotic method just described, finds that the molecular weight of hemoglobin, dissolved in distilled water, is about 32,000, while in sodium carbonate solutions it is 16,000. These results appear to show that when protein is uncombined with acids or bases it is polymerized, and so exerts a considerably smaller pressure than protein salts.

The extremely important discovery has been made by R. S. Lillie, that the osmotic pressure which is exerted by proteins (determined differentially as described) varies very pronouncedly with the nature of the inorganic acids bases or salts which their solutions contain. The following are illustrative results, obtained when dilute acids or alkalies are employed as solvents:

1.5 PER CENT. GELATIN IN DILUTE HCl SOLUTIONS.

Solvent.	Osmotic pressure of the protein in mm. Hg
Water	8.2
$^{m}/_{3100}$ HCl	6.8
$^{m}/_{2050}$ "	12.3
$^{m}/_{1550}$ "	17.9
$^{m}/_{1025}$ "	26.5
$^{m}/_{770}$ "	32.4
$^{m}/_{620}$ "	34.9
$^{m}/_{412}$ "	39.3

1.5 PER CENT. GELATIN IN DILUTE KOH SOLUTIONS.

Solvent.	Osmotic pressure of the protein in mm. Hg
Water	7.9
$^{m}/_{3100}$ KOH	14.1
$^{m}/_{620}$ "	23.7
$^{m}/_{412}$ "	25.1
$^{m}/_{310}$ "	29.0

In Lillie's words, "In the presence of either acid or alkali the osmotic pressure of the gelatin thus shows a marked increase, which, within the above range of concentrations, exhibits a certain proportionality to the quantity of acid or alkali added. For equivalent concentrations acid produces a somewhat greater increase than alkali. The change in osmotic properties is to be attributed to a finer subdivision of the colloidal particles and a consequent increase in the surface of intersection between colloidal particles and medium." The osmotic pressure of gelatin and of egg-albumin is unaffected by the addition of non-electrolytes, such as cane-sugar, glucose, glycerol and urea, but is considerably affected by the addition of **Inorganic Salts**, being depressed thereby. The decrease of the osmotic pressure exerted by the protein depends upon the nature of both the anion and the cation of the added salt. The depression increases in the order: Alkali metals < alkaline earths < heavy metals (for cations); and CNS < I < Br < NO_3 < Cl < F < plurivalent anions, SO_4, tartrate, citrate, phosphate (for anions). This fact is especially significant when we recollect that this is the order in which the various ions bring about the dehydration and coagulation of proteins (see Chapter VIII).

THE SWELLING OF PROTEIN JELLIES.

The proteins, as we have seen, exert a small, but a definite osmotic pressure. They are at the same time not diffusible through jellies or only very slightly so. Any crystalloid which may chance to be present in the external fluid which bathes a **Gelatin** plate, therefore, can penetrate the gelatin, although perhaps more slowly than water. The gelatin from the interior of the plate cannot similarly escape into the surrounding medium. The gelatin plate, therefore, acts like an osmometer which provides its own membrane which is permeable for water and crystalloids but not for colloids. Hence, when dry gelatin or concentrated jellies are placed in water they take up water and increase in volume.

A phenomenon in the domain of crystalloids which presents some analogies to the swelling of colloidal jellies is the following: If we place at the bottom of a column of distilled water a layer of **Phenol** and introduce below this a layer of saturated solution of potassium chloride in water and now allow the system to stand at constant temperature, the layer of phenol gradually moves up the column of water, in other words the layer of solution below the phenol "swells." The solvent, water, being soluble in phenol, the phenol is permeable by it, while the potassium chloride, being insoluble in phenol, cannot pass through the supernatant layer of phenol.

Not only osmotic, but also chemical phenomena must, however, play a part in the swelling of protein jellies. As we have seen in Chapter VIII the passage of a protein into solution involves the addition of the elements of water to terminal —NH_2 and —COOH groups

and also, possibly, to internal —N.HOC— -groups, resulting in the **Depolymerization** of the protein. Not only osmotic phenomena, but **Hydration** of the gelatin must, therefore, occur in the process of swelling. Confirmation of this view is afforded by the fact that the **Swelling** of gelatin is accompanied by an absorption of heat. Evidently the processes of solution and swelling are each composed of two factors, one leading to a disengagement and the other to the absorption of heat. The former process is the chemical binding of water by the protein, the latter the passage of the hydrated protein into solution (or, in swelling, the osmotic imbibition of water). In swelling, the chemical heat-effect predominates, in the dissolving of the gelatin, the heat-effect of solution.

The degree of swelling which **Gelatin** plates undergo in water is greatly enhanced by the addition of small amounts of acid or alkali to the water, the minimal imbibition of water being at a reaction very close to the neutral point. The phenomena attending the swelling of plates of gelatin in acidified water have recently been very thoroughly investigated by Procter. This investigator has found that gelatin absorbs both acid and water from acid solutions, but absorbs the acid in excess, so that the proportion of acid in the surrounding fluid diminishes. If the initial concentration of acid in the external fluid lies between 0.01 and 0.25 N, then, assuming that at the end of the process (attainment of maximal swelling) the concentration of free acid is the same within and without the jelly, having been equalized in the course of time by diffusion, the amount of acid which is "bound" by the gelatin is 0.7 to 0.8×10^{-3} ($=70$ to 80×10^{-5}) equivalents per gram. The equivalence at the attainment of maximal swelling is the same for all strong acids, but falls below this value for weak acids. While the proportion of acid which is "bound" by the gelatin varies but slightly with the concentration of the acid in the surrounding fluid, this is not true of the degree of swelling attained, which in strongly acid solutions attains its maximum at a dilution below that required for complete fixation of the acid by the gelatin, and then falls in a continuous curve with increasing concentration of the external acid solution. The same inhibition of swelling is brought about in varying degree by strong solutions of various inorganic salts, and is attributable to the dehydration of the protein which, in still stronger solutions, culminates in its **Coagulation.**

The taking up of water by gelatin from acid solutions is accounted for by Procter in the following way: He pictures the gelatin acid-compound as a coherent mass from which the gelatin molecules cannot diffuse or separate and which, in most respects, behaves like a single enormous complex molecule. It is reasonable, he considers, to visualize it as a felted mass of amino-acid chains held to each other by attractions which possibly attach only their ends, but freely admitting the passage of liquid between them. He assumes, in accordance with our older conception of the mode of formation and ionization of protein

salts, that the compound yields acid anions (for example, chlorine ions when the compound is gelatin hydrochloride), but that these anions, although diffusible, are held within the mass by the electrostatic attraction of the oppositely charged ion which, being colloidal, cannot leave the jelly in the company of its associate. The only way, therefore, in which the osmotic pressure of the anions can take effect is not by their own movement, but by the inward movement of water, resulting in the swelling of the entire jelly-mass and its dilution by admixture with the outside solution.

Two very serious objections attach to this interpretation of the phenomena. In the first place, as Procter himself has pointed out, were this the actual mechanism of swelling, then the operative force compelling movement of the water would, in ultimate analysis, be the **Electrostatic Tension** which prevents the issuance of the inorganic anions from the jelly into the solution. There should thus be a measureable difference of electrical potential between the swollen gelatin jelly and the surrounding medium. Such a difference of potential has not been found.[1] In the second place, as Procter also points out, another difficulty lies in the fact that the condition which would thus arise, would offer no equilibrium, since the concentration of the inorganic anions and the free acid itself could never become simultaneously equal within and without the jelly; no matter what degree of swelling and consequent dilution of the jelly may have occurred there will still, *ex hypothesi*, be an excess of inorganic anions within the jelly.

Our more recent views regarding the mode of formation and ionization of **Protein Salts** reconcile both these difficulties, however, for, since we now know that no inorganic ions, or at most a very small proportion, are yielded by the protein acid compound, the swelling of the jelly must be due, just as it is in the case of gelatin immersed in neutral water, to the osmotic pressure of the colloidal particles themselves, which, being unable to penetrate the colloidal network in which they are entangled, necessarily compel the compensating migration of water. No electrostatic tension between the jelly and the external solution need be assumed, because both ionic components of the protein salt are attracting water by virtue of their osmotic pressure, and they are necessarily present in the jelly in equimolecular concentration since neither of them can leave it, even in the minimal quantity necessary to create an electrostatic tension. Furthermore, since no inorganic anions are yielded by the protein salt, simultaneous equality of concentration of the uncombined acid and the acid anions within and without the jelly will be simply assured by their equal and unhampered diffusion into the jelly. The increased **Swelling-capacity** of gelatin in solutions of acids or alkalies is merely the expression of the fact that the ionization

[1] Ehrenberg, R.: Biochem. Ztschr., 1913, **53**, p. 356. Even if both of the ions of the protein salt were able to issue from the jelly, a difference of potential would arise from their unequal speeds of diffusion. See F. E. Bartell and C. D. Hocker: Jour. Am. Chem. Soc., 1916, **38**, pp. 1029 and 1036.

of the protein salt leads to an increase in the number of colloidal particles per unit volume of the jelly, and possibly, also, in part, to the fact that protein ions have a greater affinity for water than undissociated protein molecules.

This conception of the process of swelling would still yield no equilibrium or **Swelling-maximum** were there no compensating force acting in an opposite sense to the osmotic pressure of the gelatin itself. No matter how much gelatin may be diluted by swelling, there will always remain an excess of osmotic pressure within the jelly, due to the protein ions which cannot leave it. Now gelatin plates, when immersed in water or in acid solutions, do not swell indefinitely until swelling merges insensibly into solution, but, on the contrary, display a definite swelling-maximum. At this point, therefore, the osmotic pressure exerted by the colloidal particles within the jelly must be balanced by an equal opposing force, which Procter interprets as the tension of the elastic colloidal network.

The effects of **Inorganic Salts** upon the swelling of gelatin plates are complex because, as Loeb has recently demonstrated, they consist of two separate factors: In the first place a chemical interaction occurs between the gelatin and the salt, leading to the formation of a compound of the acid component of the salt with the gelatin. This compound has a greater swelling-capacity than uncombined gelatin. On the other hand the uncombined portion of the salt, when present in excess, tends in varying degree, depending upon the particular salt employed, to dehydrate the gelatin and therefore to inhibit swelling. The power of the various salts to inhibit the swelling of gelatin is proportionate to their power of coagulating proteins in solution.

The swelling of **Living Tissues** when immersed in hypotonic solutions or in acid or alkaline isotonic solutions is a very complex phenomenon. In the first place it is determined by the **Permeability** of the surfaces of the cells for water and anything affecting the permeability of the cells of the tissue will also influence the imbibition of water. In the second place the swelling of the tissue is determined by the **Osmotic Pressure** of the proteins which it contains, which is affected by acids, alkalies and salts in the manner outlined above for gelatin. In the third place degenerative changes in excised tissues such as the excised muscles of the frog's leg, lead sooner or later to production of diffusible products of **Autolysis**, and since the surface-layers of the tissue are with difficulty penetrable by some of these, their production leads to a greatly enhanced imbibition of water.

Finally, the water which is taken up by the tissue may actually enter the cells or, on the contrary, may merely be taken up into the interstitial spaces between the cells. In general it may be stated, however, that any factor tending to injure the vitality of the cells, for example heating, will greatly increase their permeability for water, and hence will increase the rate and degree of swelling in hypotonic solutions.

The fact that acids greatly increase the swelling-capacity of **Gelatin** or **Fibrin** has led M. H. Fischer to attempt to account in this way for the edematous conditions of tissues which are encountered in a variety of pathological conditions. He is of the opinion that the **Edema** of tissues is due to local development of acids which increase the affinity of the tissue-proteins for water. Many objections to this view have, however, been advanced by a number of investigators and it does not appear feasible to account for the phenomena of edema in any such simple manner. In the first place the buffer-action of the tissues and tissue-fluids must undoubtedly prevent the development of a sufficiently high acidity to account for the accumulations of fluid which occur in edema. The acidity required to influence in so decided a manner the swelling of gelatin or fibrin, is far greater than the acidity which could possibly prevail within living tissues or the tissue-fluids derived from them, and as a matter of fact very considerable edema may prevail in tissues displaying no perceptible deviation from the normal neutral or excessively faintly alkaline reaction of all living tissues and tissue-fluids. Then, again, the accumulations of fluid which occur in and characterize edema are more frequently interstitial than intercellular. The fluid is found *between* the cells and not within the cells themselves, where the proteins are present in highest concentration. It appears more probable that in the majority of the instances of edema, fluid accumulates in abnormal situations because the permeability of the membranes lining the lymph-spaces or finer bloodvessels has been increased by injury. Thus we know that various substances such as leech-extract or extracts of shell-fish or peptones, or other injurious agencies such as heating, will so greatly modify the permeability of the capillary bloodvessels as to lead to great accumulations of fluid in the lymph-spaces. Similar changes in these or other membranous surfaces may very probably account for the accumulation of fluids in the cellular interstices of tissues in certain disease-conditions. Even when edema is accompanied by the accumulation of fluid within the cells themselves, this is rather to be attributed to alterations of permeability or of the affinity of the proteins for water by disturbance of the normal balance of the inorganic salts in the protoplasm, than to local development of acidity.

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CHAPTER XIV.

PROPERTIES CONFERRED BY THE COLLOIDAL CONSTITUENTS; CHEMICAL AND BIOLOGICAL.

EFFECTS OF DISTURBANCE OF THE INORGANIC ENVIRONMENT.

We have already had occasion to discuss the effects of alteration of the total **Concentration of the Environment** upon the protoplasm inhabiting it, and we have seen that the most salient of these effects depend primarily upon the migration of water into or out of the substance of the cell. The effects arising out of alteration of the normal **Composition of the Environment** appear to have been first systematically investigated by James Blake, a physician resident in San Francisco in the decades comprised between the years 1870 and 1890. His earliest investigations upon this subject were, however, published in the *Archives générales de Médecine* and in the *Proceedings of the Royal Society of London* in 1839 and 1841 respectively; his later and more extensive investigations appeared in the *Proceedings of the California Academy of Sciences* and in the *Comptes Rendus of the French Academy of Sciences*. His inquiries into the effect of a variety of inorganic salts when injected into the circulation led to the discovery of a number of important facts; among others to the discovery of the **Anesthetic Action of Magnesium Salts** to which much importance has been ascribed in recent years. The older generation of physiologists, antedating the modern development of physical chemistry, expressed dosages and concentrations in terms of the absolute weight of substance employed and if, of two substances, a smaller weight of one was lethal than of the other that substance was deemed the more toxic of the two. It was Blake who first pointed out in physiological literature that equal weights of the various inorganic salts do not by any means contain equal numbers of molecules, and he urged that the toxicity or other physiological actions of dissolved substances be referred not to the absolute weight but to the number of gram-molecules of material administered. Proceeding upon this principle Blake was able to show that many substances hitherto considered to be of very diverse toxicity were in reality very similar in their physiological action. In particular this was found to be the case with many series of **Isomorphous Salts** of the metals. On the other hand certain metallic salts, hitherto supposed to be of like action and toxicity, were found, when tested by this new criterion, to differ very decidedly in their relative effect upon living protoplasm.

The investigations thus initiated have been continued by a very large number of subsequent observers whose labors have resulted in the accumulation of our present extensive knowledge of the influence of inorganic salts upon living matter. A large part of this information belongs more appropriately to the subject of **Pharmacology** and we will only review it here in so far as it throws an important light upon the nature of the chemical and physicochemical factors which govern the relationship of the cell to its normal environment. The detailed actions of the inorganic substances which are rarely if ever constituents of the normal inorganic environment of protoplasm we will therefore not discuss beyond the enunciation of the brief generalization that the salts of the **Heavy Metals** such as silver, copper, lead and mercury, act as corrosive poisons, leading to disintegration of the cells by coagulation and flocculation of the protoplasmic proteins, which disrupts the continuity of the gel-structure of the cell and causes it to break up into particles which fall apart, so that the substance of the cell is gradually eroded away. The compounds of **Phosphorus**, other than the phosphates, and the compounds of **Arsenic** exert peculiar effects on metabolism which are fully described in current works on pharmacology.

The discovery that the inorganic salts which form the normal constituents of the environment of cells may under certain circumstances act as protoplasmic poisons, is attributable in the first place to Ringer who, however, did not himself interpret his results in this manner. He found that if the excised hearts or skeletal muscles of cold-blooded animals be immersed in pure **Sodium Chloride** solution which is isotonic with blood serum, they lose their irritability and the power of contraction much more rapidly than they do in blood serum, or in solutions containing sodium, potassium and calcium chlorides in the proportions in which they are present in blood-serum. This was at first interpreted to mean that potassium and calcium were required by these tissues for nutritive purposes, but later investigations have clearly shown that while pure sodium chloride is definitely toxic for living tissues, its toxic properties are antagonized or annulled by a correct admixture of potassium and calcium salts.

All **Inorganic Salts** in pure solutions exert in greater or less concentrations a toxic action upon protoplasm which is evidenced by a more or less pronounced abnormality of function. In nervous or muscular tissues these effects are usually evidenced by an initial increase in irritability followed by a more or less rapid decrease and final loss of irritability. Thus, if the foot of a decapitated frog be dipped into solutions of various salts the increase of irritability of the sensory nerve endings leads to a reflex withdrawal of the foot from the solution. The following were the concentrations of various inorganic substances which Loeb found to be effective in giving rise to this reflex:

Substance.	Mineral effective concentration.
HCl	$^{m}/_{240}$
NaOH	$^{m}/_{80}$
$AgNO_3$	$^{m}/_{150}$
$FeCl_3$	$^{m}/_{60}$
$CdCl_2$, $HgCl_2$	$^{m}/_{32}$ to $^{m}/_{16}$
$AlCl_3$	$^{m}/_{16}$ to $^{m}/_{8}$
$CaCl_2$, $SrCl_2$, $BaCl_2$, $MgCl_2$, KCl, NH_4Cl, NaCl, LiCl	$^{m}/_{8}$
Sodium oxalate, Sodium citrate	$^{2}/_{3}m$ to $^{m}/_{2}$
	$^{m}/_{8}$

The effects observed were not attributable to the imbibition of water from these hypotonic solutions because, it will be recollected, the skin of the frog is not permeable to water in the direction exterior → tissues, but only in the opposite direction. The only hypertonic solutions employed, in which osmotic phenomena might have played a rôle, were those of the chlorides of the alkalies and ammonium.

It was first discovered by Biedermann in 1880 that solutions of certain **Sodium Salts** cause skeletal muscles which may be immersed in them to enter into more or less rhythmic contractions, reminiscent of those of heart-muscles in normal serum or in Ringer's solution. He also pointed out that since these contractions continue to take place in the presence of **Curare**, which paralyzes the neuromuscular junctions, the stimulus which evokes them must originate in the muscular tissues themselves, *i. e.*, the contractions are **Myogenic**. This phenomenon was reinvestigated by Loeb, who found the following minimal concentrations of the various sodium salts just sufficed to evoke the semi-rhythmic contractions in frogs' muscles, the solutions being rendered isotonic with amphibian serum by the addition of sugar or urea.

Substance.	Minimal effective concentration.
Sodium chloride	$^{m}/_{16}$
Sodium bromide	$^{m}/_{16}$ to $^{m}/_{32}$
Sodium iodide	$^{m}/_{32}$
Sodium carbonate	$^{m}/_{16}$ to $^{m}/_{32}$
Sodium sulphate	$^{m}/_{32}$
Sodium acetate	$^{m}/_{32}$ to $^{m}/_{64}$
Sodium fluoride	$^{m}/_{64}$ to $^{m}/_{96}$
Sodium formate	$^{m}/_{80}$ to $^{m}/_{160}$
Sodium oxalate	$^{m}/_{200}$ to $^{m}/_{300}$
Sodium phosphate	$^{m}/_{128}$ to $^{m}/_{256}$
Sodium citrate	$^{m}/_{200}$

It will be observed that the most efficient stimulators in this series are the salts of sodium combined with an acid (oxalic, phosphoric, citric, hydrofluoric) which precipitates **Calcium** from its solutions.

It is a very significant fact, therefore, that the addition of traces of soluble calcium salts to any of these solutions, whether containing a calcium-precipitating acid or not, results in prompt suppression of the contractions. Evidently the calcium is not required in these cases to supply a nutrient to the muscular tissues, but to antagonize an action of an excess of **Sodium** which results in abnormality of function. The simultaneous action of an excess of sodium ions and a calcium-precipitating anion is more effective than excess of sodium alone, because the calcium already present in the tissues partially antagonizes the excess of sodium in the environment. These facts led Loeb to emphasise the importance of the $\frac{Na}{Ca}$ ratio in living tissues and in their environment. Any pronounced increase in this ratio leads to hyperirritability of nervous and muscular tissues and, in fact, as Loeb has pointed out, it is only the calcium in our blood and tissue-fluids which prevents all our skeletal muscles from beating rhythmically like the heart.

The fact that the heart continues to beat rhythmically in the presence of the calcium in the blood, although the skeletal muscles cannot do so, draws our attention to the very important fact that the effect of the inorganic environment differs in different types of living tissues. This fact is very strikingly illustrated by the effects of salt solutions upon different parts of the swimming-bells of jellyfish. These bells, in normal sea-water, are almost constantly contracting in a rhythmic manner and it is by the rhythmic impetus created by the expelled water that the jellyfish propels itself through the water. It was first pointed out by the English Biologist Romanes that when the swimming-bell of the jellyfish *Gonionemus* is deprived by section of its margin, the center of the bell will no longer beat in sea-water, while the margin continues as before to beat rhythmically. Since the margin contains all the nervous ganglia of the bell Romanes concluded that the beats of the swimming-bell were initiated and regulated by these nervous tissues. Loeb, however, found that if the centers, with the margin excised, be placed in pure sodium chloride or sodium bromide solutions which are isotonic with sea-water, they will beat rhythmically just as the intact bell does in sea-water. The experiment really indicates, therefore, that the optimal salt-mixture for rhythmic excitation differs in the nervous and the contractile tissues of the bell.

Another experiment which illustrates in a very striking manner the differing effects of the inorganic environment upon tissues of diverse function is the following: When the last abdominal segment of a recently-killed fly[1] is torn out with a pair of forceps a length of intestine is usually extracted from the abdomen. The muscular tissue in the intestines of the *Insecta*, unlike ours, is striated. If this be wetted with M/6 sodium chloride solution and examined under a

[1] The species actually employed was the large Australian "bluebottle," *Callophora villosa*.

microscope, rhythmic contractions will be seen travelling in rapid succession from the upper to the lower end of the intestine. On touching the intestine at about the middle point with a finely pointed camel's-hair brush wetted with M/6 calcium chloride solution the rhythmic contractions in the affected area are immediately suppressed, but on following a wave of contraction with the eye as it enters this area and disappears, it can be seen to re-issue below the affected area at the moment when it would have appeared, had the contraction actually traversed that section of the intestine. Evidently, while the contractile function has been suppressed, the conductive functions of the tissues are still unimpaired. If, however, the middle part of the intestine is touched with potassium chloride solution instead of calcium chloride solution, both conduction and contraction are suppressed and rhythmic contractions remain confined to the region above the affected area, the region below remaining quiescent. Conduction and contraction are therefore very diversely affected by these inorganic salts.

EFFECTS OF REMOVAL OF CALCIUM FROM THE TISSUES AND TISSUE-FLUID.

We have seen that an increase in the $\frac{Na}{Ca}$ ratio in excised muscular tissues leads to hyperirritability of the tissues and that the ratio may be increased in either of two ways, namely by increasing the concentration of the **Sodium Ions** in the environment or by decreasing the **Calcium Ions** by employing a salt of which the acid component either precipitates calcium or forms a sparingly dissociated compound with it.

The effects of injection of calcium precipitants such as citrates, oxalates, fluorides, tartrates, oleates and other soaps, etc., are very widespread and fundamental. They are traceable to muscular, nervous and glandular tissues. In small doses whether taken by mouth or injected intravenously, they act as **Cathartics**, inducing enhanced peristalsis and the evacuation of fluid feces. In larger doses we obtain, in addition to purgation, peripheral twitchings, *i. e.*, irregular involuntary contractions of the muscles of the extremities. An effect upon the renal epithelium is also evidenced by a marked **Diuresis**, or abnormal volume and dilution of the urine. In still larger doses a very curious complex of symptoms is elicited. Shortly after the injection of massive doses of sodium citrate subcutaneously in rabbits, peripheral twitchings occur which are rapidly succeeded by convulsive movements and marked disturbances of equilibrium. The forelegs are stiffly extended and continually shuffling forward with a motion resembling an effort to maintain equilibrium upon a slippery or moving surface. The head is thrown back and the jaws are continuously chewing. Not infrequently the animals throw themselves into backward somersaults. Exactly the same effects, without the peripheral twitchings, purgation or diuresis, are obtained if, instead of administering massive doses to

the whole animal, minute doses are applied to the **White** matter of the **Cerebellum**, by direct injection below the gray matter of the surface. The convulsive effects of large doses are therefore attributable to excitation of the white matter of the cerebellum. *Chronic* ingestion or injection of calcium precipitants leads to partial solution of the bones which become thin and soft, a condition not infrequently met with in sheep that have been feeding for some time upon plants of the *Oxalis* group. The effects of calcium precipitants in the order in which they occur are therefore: 1. Purgation. 2. Peripheral muscular twitchings and diuresis, 3. Cerebellar excitation. 4. Partial solution of the bones. A measure of **Tolerance** to the first three groups of effects is developed after repeated administration.

The variety of sensitiveness and response of differing tissues to modifications of the inorganic environment is again displayed in these effects. The very striking instance of the varying sensibility of different cells to this type of environmental disturbance is afforded by the complete insensitiveness of the **Gray Matter** of the central nervous system to calcium precipitants. We have noted above that the cerebellar effects of calcium precipitants are only elicited when the salt reaches the white matter of the cerebellum, and Maxwell has shown that solutions of the various calcium precipitants are without effect when placed upon the surface of the motor-area of the cerebrum, but immediately induce convulsions when they penetrate by diffusion or injection to the underlying white fibers.

The origin of the purgation by the **Saline Cathartics** has been the subject of much and prolonged discussion. The earliest suggestion was that made by Poiseuille and Liebig, to the effect that the action of these cathartics was a purely osmotic one; the excess of salt within the intestinal cavity withdrawing water from the tissues and tissue-fluids, while the tension of the intestinal musculature caused by this collection of fluid within the lumen of the intestine led to rapid expulsion of the contents. The great French Physiologist, Claude Bernard, however, showed that the *intravenous* injection of sulphates caused purgation, although the osmotic effect in this instance should be the reverse of that imagined by Poiseuille and Liebig, and water should be withdrawn from the intestine into the circulation. To meet this objection a modification of the osmotic theory was subsequently brought forward by Wagner and Schmiedeberg, who suggested that the saline cathartics modify the **Permeability** of the intestinal epithelium, in such a manner that the absorption of water from the intestine is hindered and the fluidity of the contents and distention of the muscular walls, which ensues from the accumulation of unabsorbed fluids, leads to the rapid evacuation of fluid feces.

The discovery by Loeb that those salts which increase the $\frac{Na}{Ca}$ ratio, and especially those which precipitate calcium, induce hyperirritability in muscular tissues, at once threw a new light upon the action of the saline cathartics. A large proportion of these cathartics are

sodium or magnesium salts of acids such as sulphuric, carbonic, phosphoric, citric or tartaric acids which form insoluble or sparingly dissociated compounds with calcium, and their action in stimulating the muscles of the intestine may be regarded simply as an instance of a general effect upon contractile tissues. **Barium Chloride**, which stands in the peculiar position of not being a calcium precipitant, and nevertheless being a powerful stimulant of muscular tissues, is also a very drastic purgative. The inorganic reagents which induce contractions in excised skeletal muscles, therefore, cause purgation when administered by mouth or injected intravenously.

Continuing and extending the above-cited investigations of Claude Bernard, J. B. Macallum showed that if 10 c.c. doses of M/6 sodium citrate, sulphate or tartrate be administered subcutaneously to rabbits, followed ten minutes later by 5 c.c., and ten minutes after that by 5 c.c. more, very pronounced purgation follows. Purgation may also be induced by perfusion of these solutions into the bloodvessels supplying a loop of intestine, or even by painting the solution upon the peritoneal surface of the intestine. By whatever avenue the salt reaches the muscular tissue, therefore, contractions are induced. This disposes of the original osmotic theory. The theory of Wagner and Schmiedeberg, that the fluidity of the feces induced by these salts is due to the non-absorption of water from the intestine, was shown to be unnecessary by the discovery by Macallum that part, at least, of the fluidity of the feces is attributable to the active secretion of fluid from the mucous glands of the intestine into its lumen. Thus, a loop of intestine about 30 centimeters long in a rabbit was thoroughly cleaned out by compression and the ends tied. From time to time before and after the administration of a cathartic salt the loop was opened and the content of fluid determined. The following is an illustrative result:

Loop contained at the beginning	5.0 c.c.
Fluid secreted in the first ten minutes	0.2 "
Fluid secreted in the second ten minutes	0.5 "
2 c.c. of $^{m}/_{8}$ barium chloride injected subcutaneously.	
Fluid secreted in the first ten minutes after injection	4.0 c.c.
Fluid secreted in the second ten minutes after injection . . .	3.4 "
Fluid secreted in the third ten minutes after injection	3.0 "

the loop after the administration of the barium chloride, also underwent powerful contractions.

Even when the saline cathartic is administered by mouth, the operative portion of it is that which reaches the intestine through the medium of the circulation, so that even in this instance an osmotic effect of the salt is excluded. This has been very strikingly shown by the experiments of Hertz, Cook and Schlesinger, conducted in Guy's Hospital in London. These observers employed human subjects for their experiments, following the passage of the cathartic down the intestine by simultaneously administering bismuth oxychloride and following the shadow cast by this substance on an *x*-ray plate. Sepa-

rate experiments upon a patient with an iliac fistula showed that the cathartic and the bismuth oxychloride travelled down the intestine together, *i. e.*, the cathartic did not reach any point of the intestine in appreciable advance of the shadow cast upon the plate.

Three persons received two ounces each of bismuth oxychloride in half a pint of cold water at 8 A.M. Breakfast was given at 8.30. Cecal sounds were heard and a shadow appeared in the cecum four hours after the meal.

Two days later the same persons received a Seidlitz powder with the same mixture. The shadow appeared in the cecum at the usual time, namely, four hours after the meal, but while normal feces were passed before breakfast, fluid stools, due to the cathartic, were passed at 9.15, 9.40 and 9.45 respectively, no less than three hours before the first trace of bismuth or of the saline cathartic reached the cecum by the way of direct passage through the intestine.

The same conclusion was reached by chemical analysis of the feces, a sulphate being in this instance employed as the cathartic salt:

Feces.	Per cent. of water.	Total SO_4	Per cent. of SO_4.
First day normal	80.9	0.037	0.045
Second day normal (10.15) . . .	80.0	0.016	0.032
Second day watery (11.25) . . .	91.1	0.091	0.041
Third day normal	77.3	0.270	0.220

Thus the watery feces evacuated in response to the cathartic contained very little more sulphate than the normal feces of the preceding day, while the normal feces of the day following the purgation contained less than the normal percentage of water, and a great excess of sulphates. Were either Liebig's or Wagner and Schmiedeberg's hypothesis the correct interpretation of the facts, we would expect these feces to be very fluid, whereas the experiment shows that the sulphate that remains unabsorbed is actually much less efficient in promoting peristalsis than the proportion which circulates in the blood-stream. That an excess of sulphates were actually circulating in the blood-stream while purgation was taking place is evidenced by the fact that the urine collected between 8 A.M. and 4 P.M. on the second day contained 0.624 grammes more SO_4 than the urine collected during the same period on the previous day.

It must be admitted that the purgative action of the saline cathartics is not to be entirely accounted for by the precipitation of the calcium in the tissues, since **Barium** and **Magnesium**, irrespective of whether they are combined with calcium precipitating acids or not, will induce purgation. The possibility must be borne in mind, however, although we as yet possess no direct evidence which bears upon it, that barium and magnesium, being related divalent metals, may possibly displace calcium from certain compounds in the protoplasm of the tisues affected, and in this connection it is perhaps significant that the urinary output of calcium runs parallel to the output of

magnesium. Furthermore both the cathartic and the anesthetic actions of magnesium salts are antagonized and annulled by the administration of calcium salts. At all events barium salts share with the calcium precipitants the common property of inducing hyperirritability in muscular tissues, while the exceptional sensitiveness of the intestinal musculature to magnesium may be perhaps regarded as affording another instance of the diverse susceptibility of the various types of tissue-cells to the influence of changes in the inorganic environment. That the intestinal musculature is not the only tissue which is profoundly affected by magnesium salts is shown by the fact that the introduction of a considerable excess of magnesium chloride into the blood-stream induces **Glycosuria** in rabbits.

THE MUTUALLY ANTAGONISTIC ACTION OF SALTS, AND PHYSIOLOGICALLY BALANCED SOLUTIONS.

We have already seen that a small proportion of calcium inhibits the action of sodium salts in inducing rhythmic pulsations of many contractile tissues. This is, however, merely a particular instance of a very general phenomenon, as the investigations of Loeb in animal physiology and of Osterhout in plant-physiology have most abundantly demonstrated.

For example, the fertilized eggs of the marine fish *Fundulus* will develop normally in distilled water. Inorganic salts are therefore not necessary for their nutrition. They will also develop normally, of course, in sea-water in which the various saline constituents other than bicarbonates and phosphates are present in approximately the following concentration and proportion (Van t'Hoff's solution):

5/8m sodium chloride	1000	parts by	volume
5/8m magnesium chloride	78	"	"
5/8m magnesium sulphate	38	"	"
5/8m potassium chloride	22	"	"
5/8m calcium chloride	10	"	"

In $\frac{5}{8}$ m sodium chloride solution, without the admixture of the other salts, however, the eggs will not live for more than twelve hours, despite the fact that this solution is isotonic with sea-water. Evidently sodium chloride is definitely toxic for these organisms. In the following mixtures:

96 c.c. of 5/8m NaCl + 4 c.c. of 5/8m $MgCl_2$
96 c.c. of 5/8m NaCl + 4 c.c. of 5/8m KCl
96 c.c. of 5/8m NaCl + 4 c.c. of 5/8m $CaCl_2$

they will live only for about twenty-four hours or even less. When the eggs are placed in the following solutions:

96 c.c. of 5/8m NaCl + 2 c.c. of 5/8m $MgCl_2$ + 2 c.c. of 5/8m $CaCl_2$
96 c.c. of 5/8m NaCl + 2 c.c. of 5/8m $MgCl_2$ + 2 c.c. of 5/8m KCl
96 c.c. of 5/8m NaCl + 2 c.c. of 5/8m $CaCl_2$ + 2 c.c. of 5/8m KCl

the eggs live for less than thirty days in the first two solutions, but in the third, which corresponds in composition with a concentrated **Ringer's Solution**, the eggs live for an indefinite period and develop normally. Evidently the toxic properties of sodium chloride are neutralized or antagonized by admixture with a small proportion of other inorganic salts. When the correct mixture is obtained the solution is devoid of toxicity and we speak of it as a "Physiologically Balanced" salt solution. Evidently Ringer's solution and sea-water are physiologically balanced solutions in so far as the tissues of *Fundulus* are concerned.

Sodium chloride is not peculiar in exerting a toxic effect in pure solution. In fact it may be said that any salt without admixture with other salts is more or less toxic for living protoplasm. This is very clearly demonstrated by the following among very many experiments of this character which we owe to Loeb.

Antagonism between sodium chloride and zinc sulphate (*Fundulus*).

Solution.	Percentage of eggs which develop:
100 c.c. H_2O	49
100 c.c. H_2O + 8 c.c. 1/32m $ZnSO_4$	0
100 c.c. 8/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	1
100 c.c. 7/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	6
100 c.c. 6/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	8
100 c.c. 5/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	29
100 c.c. 4/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	34
100 c.c. 3/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	37
100 c.c. 2/8m NaCl + 8 c.c. 1/32m $ZnSO_4$	38

Evidently a very dilute solution of zinc sulphate is highly toxic for *Fundulus* eggs. Sodium chloride in excess is also very toxic. A definite admixture of these two toxic salts may be found, however, which is almost devoid of toxicity.

In the above experiment we have an instance of antagonism between a monovalent metal and a divalent metal. Antagonism may also be displayed between two monovalent metals or between two divalent metals. The following is an illustrative example:

Antagonism between magnesium chloride and strontium chloride (*Fundulus*).

Solution.	Percentage of eggs which develop:
100 c.c. 5/8m $MgCl_2$	0
100 c.c. 5/8m $MgCl_2$ + 1 c.c. of 5/8m $SrCl_2$	25
100 c.c. 5/8m $MgCl_2$ + 2 c.c. of 5/8m $SrCl_2$	22
100 c.c. 5/8m $MgCl_2$ + 3 c.c. of 5/8m $SrCl_2$	9
100 c.c. 5/8m $MgCl_2$ + 4 c.c. of 5/8m $SrCl_2$	0
100 c.c. 5/8m $MgCl_2$ + 5 c.c. of 5/8m $SrCl_2$	0

Similar antagonism was found to subsist between lithium chloride and zinc sulphate, potassium chloride and zinc sulphate, ammonium chloride and zinc sulphate, sodium acetate and lead acetate, sodium chloride and manganese chloride, sodium chloride and cobalt chloride,

sodium chloride and lead acetate, sodium chloride and aluminium sulphate, sodium chloride and chromium sulphate, potassium chloride and calcium nitrate and, in fact, some measure of mutual antagonism is usually but not invariably found to subsist between every pair of inorganic salts.

Very striking examples of the mutual antagonism of inorganic salts are afforded by the experiments of Osterhout upon plant-tissues. The following shows the aggregate length of roots produced after sixty days by wheat-seeds allowed to germinate in various salt solutions of $\frac{3}{25}$ molecular concentration.

Solution.	Aggregate length of roots mm.
Sodium chloride	59
Potassium chloride	68
Magnesium chloride	7
Calcium chloride	70
1000 NaCl + 10$CaCl_2$	254
1000 NaCl + 22KCl + 10$CaCl_2$	324
1000 NaCl + 78$MgCl_2$ + 10$CaCl_2$	377
1000 NaCl + 78$MgCl_2$ + 38$MgSO_4$ + 22KCl + 10$CaCl_2$	360
Distilled water	740

Since the roots exhibit a maximum growth in distilled water the various salts are evidently not required for the *nutrition* of the plants. The individual salts in pure solution are all highly toxic as compared with distilled water, but mixtures of the salts in proportions approximating to those found in sea-water permit very extensive growth of roots to occur. The following data show the percentage-increase in the length of the thallus which develops from the seeds of *Equisetum* in various salt solutions of $\frac{3}{160}$ molecular concentration.

Solution.	Percentage increase in length of thallus after 50 days.
Sodium chloride	0
Potassium chloride	0
Magnesium chloride	0
Calcium chloride	700
1000NaCl + 22KCl	0
1000NaCl + 78$MgCl_2$	40
1000NaCl + 78$MgCl_2$ + 22KCl	40
1000NaCl + 10$CaCl_2$	980
1000NaCl + 22KCl + 10$CaCl_2$	1500
1000NaCl + 78$MgCl_2$ + 10$CaCl_2$	1760
1000NaCl + 78$MgCl_2$ + 38$MgSO_4$ + 22KCl + 10$CaCl_2$	1500
Distilled water	1760

In certain instances the toxicity of such a universally distributed substance as sodium chloride may be extremely great. Thus Osterhout found specimens of *Vaucheria* which were killed within a few days by so small a concentration as $\frac{m}{10000}$ sodium chloride, although the running water in which these algæ were growing contained no less than twelve times this concentration of sodium chloride. In the brook, however, the toxicity of the sodium chloride was completely annulled by the

traces of other salts, magnesium, potassium and calcium chlorides which the water contained.

Similar phenomena of antagonism have been observed by C. B. Lipman in culture-media containing bacteria. In certain cases, however, no mutual antagonism was observed, as in the case of magnesium and calcium salts acting upon *Bacillus subtilis*. Furthermore, although the toxicities of potassium chloride and calcium chloride for this, and probably for other am nonifying bacteria, are mutually diminished by their admixture, this is not the case for sodium and calcium chlorides, a mixture of these two salts being more toxic for all proportions of calcium than sodium chloride alone. These exceptional phenomena appear to differentiate the ammonifying bacteria very sharply from other types of living tissue.

The mutually antagonistic toxicity of inorganic salts is therefore a phenomenon which is universally displayed whatever type of protoplasm we employ. In certain types, as those afforded by the ammonifying bacteria, certain antagonisms may fail to be exhibited, but other pairs of salts, again, will clearly annul each other's toxicity. We may infer therefore, that the toxicity of pure salts for protoplasm is a universal property, and that in the majority of instances a mixture of any two salts is less toxic than either of the components alone. It is certainly not an accident that for all the forms of life which have been investigated, the most nearly innocuous mixtures correspond in composition either to sea-water (Van t'Hoff's solution) or to Ringer's solution. In these mixtures of five and three salts respectively the annulment of toxicity is far more complete than in any binary mixture. Sea-water and Ringer's solution are therefore, **Physiologically Balanced Solutions**, but for certain of the higher animals, for example in the mammals of which the tissues are adapted to an environment having the composition of Ringer's solution, sea-water, as it is composed today, is no longer a physiologically balanced solution. The determination of the physiological balance depends, therefore, upon the properties of the protoplasm upon which the salts are acting and not upon any peculiar properties of the salt-mixture in question, such as double salt-formation, etc.

THE ORIGIN OF THE MUTUAL ANTAGONISM OF INORGANIC SALTS.

Since the mutual antagonism of salts originates in a property of protoplasm rather than in any physical peculiarity of the salt-mixtures, we are led to infer that the phenomenon must probably be due to chemical interactions between the constituents of the salt-mixture and some constituents of the cells. Now antagonism, as we have seen, may be displayed between almost any pair of metal ions, but it may also be displayed between different pairs of acid radicals. Moreover the toxicity of both acids and bases may be partially annulled by

suitable neutral salts. It is clear, therefore, that any constituent of the cell which is responsible for these phenomena must be capable of entering into combination with both acids and bases and with both the acid and the basic radicals of salts. The **Proteins** are the only abundant constituents of the cell which have been demonstrated to possess these properties, and it has therefore been inferred by Loeb and is now very generally assumed, that the toxicity of sodium salts, for example, is attributable to the formation of sodium proteinates which, if present in too great a proportion in the cell, confer upon the protoplasm properties which are incompatible with the maintenance of normal function. The toxicity of calcium salts is regarded as being attributable in like manner to the undue predominance of calcium proteinates in the cell. An admixture of several types of protein salts is requisite to confer upon the protoplasm of the cell the exact complex of qualities essential to the maximal furtherance of its vital activities.

Much light has been thrown upon this question by two very striking series of investigations, namely the **Flotation Experiments** of Loeb and the **Conductivity Experiments** of Osterhout. The eggs of the marine fish *Fundulus* which were employed in the earlier experiments cited above have a specific gravity which is considerably in excess of that of sea-water. They will float in a $\frac{1.2}{8}$ molecular solution of sodium chloride, while they sink in a $\frac{1.1}{8}$ molecular solution. The experiments consisted in placing the eggs in solutions exceeding $\frac{1.2}{8}$ molecular in concentration, which is, of course, considerably hypertonic to the contents of the eggs, and observing how long they will float in such solutions. The withdrawal of water from the eggs is manifested not only by shrinkage of volume, but by a coincident increase in specific gravity which results finally in the eggs acquiring a higher specific gravity than the medium so that they sink in it. Continued flotation in hypertonic solutions is therefore evidence of impermeability of the superficies of the cell for water.

It is found that if the eggs are placed in a 3 molecular solution of **Sodium Chloride** they will float, but as a rule not longer than three hours. After that they sink to the bottom of the test-tube while the loss of water which has led to their sinking is evidenced by collapse of the egg-membrane, and shrinkage of the yolk-sac. When the eggs are placed in a $\frac{1.0}{8}$ molecular solution of **Calcium Chloride** they float at first, but they sink in about half an hour. If, however, the eggs are placed in a mixture of 50 c.c. of 3 molecular sodium chloride and 2 c.c. of $\frac{1.0}{8}$ molecular calcium chloride, they will float for three days or more at the surface of the solution, the eggs shrink but little or not at all, and the embryos continue to live. In a mixture of 50 c.c. of $2\frac{1}{2}$ m. NaCl + 1 c.c. of $2\frac{1}{2}$ m. KCl + 0.75 c.c. of $2\frac{1}{2}$ m. $CaCl_2$ some of the eggs will continue to float for as long as ten days, while in a $2\frac{1}{2}$ m. solution of pure sodium chloride they do not float for more than a few hours.

These phenomena admit of only one explanation, namely, that in normal sea-water the superficies of the *Fundulus* egg is practically

impermeable to water, but that in a physiologically unbalanced salt solution this natural impermeability is lost, and hence, if the solution is at the same time hypertonic, water diffuses out of the egg and the resultant increase in specific gravity causes it to sink. The same solutions which cause this loss of water are also toxic for the developing embryos. Hence the *toxicity of unbalanced solutions is associated with an increased permeability of the cells.*

The same conclusion has been reached by Osterhout in quite another way. This observer has employed the electrical conductivity of plant-tissues as a measure of their permeability for ions, that is of the resistance which the surfaces of the cells offer to the transport of ions across them. Discs about 13 m.m. in diameter were cut from the fronds of marine algæ (*Laminaria*), the average thickness of a frond being about 0.5 m.m. One or two hundred of these discs were then packed together like a roll of coins, into a solid cylinder of from 50 m.m. to 100 m.m. in length. They were held in place by glass rods so arranged as to make a hollow cylinder which closely fitted over the outside of the solid cylinder of tissue. The spaces between the rods allowed free access of various salt solutions to the living tissue. At each end of the cylinder of tissue was placed a platinum electrode which could be pressed firmly by means of a screw against the opposite ends of the cylinder. The conductivity of the cylinder was estimated in the usual way. The surface in and out of which ions were forced by the current, amounted to from 26,000 to 53,000 square centimeters; an increase in the conductivity of the cylinder implied decreased resistance to the passage of ions across the surfaces of the tissue, *i. e.*, an increased permeability for electrolytes, while a decrease in the conductivity of the cylinder implied, on the contrary, decreased permeability of the cells. It will be observed that the permeability measured by Osterhout was permeability for dissolved electrolytes, while that measured by Loeb was permeability for water.

On transferring the cylinder of *Laminaria* from sea-water to **Sodium Chloride** solution of the same temperature and conductivity (0.52 molecular), the resistance fell from the initial value of 1100 ohms in sea-water to 890 ohms in ten minutes. In fifteen minutes it had fallen to 780 ohms, after sixty minutes to 420 ohms, and thereafter continued to fall steadily until it reached a constant minimal value of 320 ohms, which was found to be the resistance of a column of sea-water of the same length and diameter. In other words, in pure sodium chloride solution the cell-surfaces in *Laminaria* increase in permeability until finally they interpose no resistance at all to the transference of ions across them.

A very striking contrast to this result is obtained if a similar column of tissue be transferred from sea-water to a solution of **Calcium Chloride** having the same conductivity as sea-water. In this case the resistance of the tissue initially *rises*, very often from the initial sea-water value of 1100 ohms to 1750 ohms in the first fifteen minutes. The resistance

remains stationary at this level for some hours, and then slowly sinks until it finally reaches the level of 320 ohms, which represents zero resistance on the part of the surfaces within the tissue. The permeability of the cell-surfaces in calcium chloride solutions, therefore, at first decreases and later increases.

A mixture of 1000 c.c. of molecular sodium chloride + 15 c.c. of molecular calcium chloride was then diluted until it had the same conductivity as sea-water. A similar column of *Laminaria* tissue, when placed in this mixture, neither gained nor lost resistance, and had the same conductivity after twenty-four hours as it normally has in sea-water. The antagonistic action of calcium chloride upon the toxicity of sodium chloride is therefore seen to depend upon the maintenance of the normal permeability of the cells.

In general it was found that while the salts of monovalent cations such as sodium, potassium, cæsium, rubidium, lithium and ammonium increase the permeability of the tissue from the beginning, the salts of divalent cations, such as magnesium, calcium, barium, strontium, manganese, cobalt, iron, nickel, zinc, cadmium and tin agree in bringing about an initial decrease of conductivity followed by a relatively gradual increase. The initial decrease is, however, very slight in the case of magnesium. Acids resemble the divalent cations in causing an initial decrease followed by an increase of permeability, but both the increase and the decrease are much more rapid than they are in solutions of neutral salts of divalent cations. Alkalies resemble the salts of the monovalent cations in causing an increase of permeability from the first.

If the increase in permeability does not exceed a certain limit, the return of the tissue to normal sea-water results in the restoration of normal permeability and the tissue is not permanently injured. If, however, the increase of permeability exceeds this limit then the normal permeability is not recoverable and the attainment of absolute permeability, *i. e.*, zero resistance of the surfaces of the cells to the passage of electrolytes across them, indicates death of the tissue. The toxic action of pure salts is therefore seen to originate in the irreparable impairment of the normal resistance which the surface of the cell opposes to the penetration or exit of water and inorganic salts.

Having regard to the fact that the **Proteins** of the cell are the only abundant constituents which are capable of entering into combination with all of these diverse substances we may assume that the alterations of **Permeability** which attend immersion of living tissue in abnormal inorganic environments are due to alterations in the physical consistency of the interstitial protein solution or jelly which holds the lipoidal elements in suspension. Alterations in the consistency of the **Interstitial Protein Medium**, and especially alteration of the texture of the spongework of which it is composed, must necessarily modify the spacing of the superficial lipoidal elements, and by widening or narrowing the interstitial pores, increase or decrease the permeability of the

cell for water and for substances which are soluble in water, but insoluble in fats.

Of course the alteration of the texture of the interstitial protein jelly which ensues when cells are immersed in abnormal inorganic media may be expected, not only to affect the permeability of the cells, but also a variety of other properties of the cells, and in this way to affect a variety of their functions. Thus, as Loeb has pointed out, the effects of diverse salt solutions, and especially those of calcium precipitants upon the phenomena of motility, are not solely and directly to be attributed to changes in the permeability of the superficies of the contractile elements. Indeed it would be manifestly unreasonable on *a priori* grounds to make such an assumption. The permeability of the cells having been affected, however, the salts which penetrate them induce further changes which modify their performance of function. This is very clearly indicated by the following experiments in which Loeb sought to ascertain whether the ratio of $\frac{Na}{Ca}$ or of $\frac{Na + K}{Mg + Ca}$ which is requisite for the maintenance of life is the same as that required for the maintenance of motility. The eggs of *Fundulus* were immersed in solutions of sodium chloride of varying concentration, and the concentration of calcium chloride which had to be added to each sodium chloride solution to permit fifty per cent. of embryos to form was determined. It was found that if the concentration of sodium chloride varies in the ratio 1 : 2 : 3 the requisite additions of calcium chloride vary in the proportion 0.3 : 1.3 : 3.2. In other words, if we double the concentration of sodium chloride we must quadruple the amount of calcium chloride, and if we triple the concentration of sodium chloride we must add about ten times as much calcium chloride. To permit normal development and therefore, presumably, to maintain normal **Permeability** calcium chloride must be added almost in the ratio of the *square* of the concentration of the sodium chloride.

Now when we turn to the proportion of calcium necessary for the maintenance of unimpaired **Motility** we find a very different relationship obtaining. For this investigation the newly hatched larvæ of a barnacle (*Balanus eburneus*) were employed. These larvæ are incessant swimmers, and they rise to the surface of the water. They are able to live in sea-water varying in concentration from $^1/_{16}$ m to $^6/_8$ m. When the larvæ are put into a pure solution of NaCl+KCl in the proportions in which these two salts exist in sea-water, they will all fall to the bottom of the vessel which contains them. They are unable to swim, although they may live for a number of hours in such a solution. If one salt with a bivalent cation be added, for example $CaCl_2$ or $SrCl_2$ in sufficient quantity, they will rise to the surface but they cannot stay there very long. If, however, enough of a *mixture* of $CaCl_2+MgCl_2$ is added, in the proportions in which calcium and magnesium are present in sea-water, the larvæ will rise to the surface and remain there, constantly swimming. Various concentrations of the Na+K mixture were employed and the concentration of bivalent cations, Mg+Ca, required to preserve motil-

ity in each solution was determined. The results showed that the ratio $\frac{Na + K}{Mg + Ca}$ was constant over a wide diversity of concentrations. In other words the concentration of bivalent ions necessary to preserve motility varied *directly* as the concentration of monovalent ions and not as the square, as in the case of permeability. While motility, therefore, is affected by changes in permeability, the effects upon motility involve changes which are not identical with those which underlie the alterations of permeability.

The permeability of the surface of the cell for substances dissolved in water is presumably determined by the diameter of the interstitial pores filled with protein jelly which comprise the spaces between the lipoidal elements of the superficial emulsion. We have seen that permeability is affected by reagents which presumably affect the solubility or state of aggregation of the protein constituents of the cell. We should expect, however, to find the permeability of the surface of the cell also affected by lipoid-solvents, especially if these enter into the lipoidal droplets and increase their diameter.

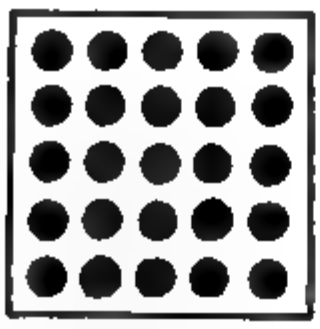

FIG. 18.—Showing successive effects of increasing diameter of the oil-droplets in an emulsion upon the size of the interstitial spaces. As the droplets increase in size until they touch each other the area of the interstitial spaces diminishes. Further increase in the diameter of the oil-droplets increases the sectional area of the interstitial spaces.

According to the measurements of Osterhout, the various lipoid-solvents, in particular, ether, chloroform, chloral hydrate and alcohol which are also **Anesthetics,** exert two effects upon protoplasm: The one consists in a decrease of permeability which is reversible, *i. e.*, disappears after removal of the anesthetic. The other effect, which requires large dosages, in an increase of permeability which is found to be irreversible. Since anesthesia is reversible we may presume it to be associated with the former of these effects while the ultimate toxic or lethal effects of these drugs may be referred to the irreversible increase of permeability.

The absorption of these substances by the lipoidal elements of the superficial emulsion with consequent increase in the volume of the lipoidal droplets might lead either to decreased or increased permeability for substances which are soluble in water. Provided the lipoidal droplets are not, in the normal superficies of the egg, in physical contact with one another, the interstitial spaces between the droplets will

be *reduced* in diameter by the swelling of the droplets. As soon as the droplets come to touch one another, however, any further increase in their diameter will push their peripheries further apart and increase the diameter of the interstitial pores.

This will be clear from the accompanying diagram depicting the three conditions indicated (Fig. 18). It can readily be seen, therefore, how a lipoid-solvent may, in small doses, decrease the permeability of cells for water-soluble substances and, in larger doses, increase it.

THE ORIGIN OF ACID SECRETIONS.

It has always been, until within very recent years, a fact exceedingly puzzling to physiologists that certain secretory glands, particularly the glands of the gastric mucosa and the "salivary" glands of carnivorous molluscs, elaborate a strongly acid secretion from an alkaline medium, namely blood or other tissue-fluids. The alkalinity of the medium was, of course, greatly overestimated by the earlier observers. On the other hand, however, the results of the most exact measurements show that the blood and tissue-fluids are on the alkaline side of neutrality, while the acidity of gastric juice, of which the components must in the long run have been derived from the blood, is comparable with that of a hundredth-molecular solution of hydrochloric acid.

The first hypothesis which was advanced in explanation of this phenomenon is usually but erroneously attributed to the German biological chemist, Maly, who published it in 1874. It actually originated with an American, E. N. Horsford whose account of this hypothesis is contained in an article contributed to the Proceedings of the Royal Society of London in 1869. He observed that if a mixture of neutral or even weakly alkaline salts, such as the **Phosphates**, be enclosed within a parchment-membrane and allowed to diffuse through it into distilled water, the water outside the membrane becomes acid in reaction, while that within the membrane becomes correspondingly more alkaline. This phenomenon is due to the fact that the diffusion-velocity of acids is more rapid than that of the alkaline salts which are formed within the dialyzer. From the alkaline blood, containing chlorides and phosphates, therefore, the acid hydrochloric juice was supposed to arise in an analogous manner. The difficulty which confronts this hypothesis is, however, that it proves too much, since by parity of reasoning all the secretions of the tissues should be acid in reaction, whereas, as a matter of fact, the majority of the secretions resemble the blood in reaction or else, as in the case of the pancreatic juice, are actually more alkaline than the blood. Moreover the effects observed in the dialysis of salt mixtures are too small in magnitude to account for the relatively high acidity of gastric juice. An alternative hypothesis advanced by Koeppe is even more difficult of acceptance. This investigator supposes that the gastric mucosa is permeable to sodium ions but not for chlorine ions. As sodium ions in the food

leave the stomach and penetrate the tissues an equivalent number of hydrogen ions migrate from the tissues into the lumen of the stomach and there combine with the chlorine ions to form **Hydrochloric Acid.** In the first place the assumption of the differential permeability of the stomach-wall for sodium and hydrogen ions is purely gratuitous and has no foundation in direct observation, and in the second place the theory would require the presence of food in the stomach before acid gastric juice could be secreted, whereas, as Pavlov has shown, the secretion of acid gastric juice may be excited reflexly without the presence of any foodstuffs in the stomach.

T. B. Osborne has, however, drawn attention to a mechanism whereby an acid fluid may be elaborated through the intermediation of **Proteins.** When **Edestin** is dissolved in sodium chloride solutions and then precipitated by passing in a stream of carbon dioxide, it is found that the precipitate contains an excess of combined hydrochloric acid, while, on the other hand, an equivalent mass of sodium carbonate or bicarbonate has been formed in the fluid and may be estimated by titration with methyl orange. When, in other words, the excess of sodium hydroxide is neutralized by carbon dioxide, this protein compound of sodium chloride breaks up, setting free sodium hydroxide and retaining hydrochloric acid in combination. A precisely similar phenomenon occurs when **Red Blood-corpuscles** are repeatedly washed with isotonic salt solution until the washings become perfectly neutral and are then suspended in neutral sodium chloride solution and treated with a stream of carbon dioxide. The external fluid becomes alkaline and the blood-corpuscles become richer in chlorine (Gürber). In this way hydrochloric acid is brought into combination with a non-diffusible base, and may be subsequently separated from it by hydrolytic dissociation, followed by the diffusion of the hydrochloric acid into the surrounding medium, or in the particular instance under consideration, into the gastric juice. We may infer, therefore, that the secretion of an acid juice depends upon the existence in the secreting cells of a protein which is capable of decomposing sodium chloride in the presence of carbon dioxide. The appearance of the free hydrochloric acid in the secretion being attributable to the colloidal, indiffusible character of the protein base.

THE SELECTIVE ACTION OF TISSUES AND THE "OLIGODYNAMIC" ACTIONS OF HEAVY METALS.

It is a universal phenomenon in living tissues that despite the fact that the exact composition of the inorganic milieu is so definitely related to their welfare and can depart so little from normality without inducing disturbances of permeability, yet the relative proportions of the various inorganic constituents of the protoplasm do not conform at all to the proportions subsisting in the medium which they inhabit.

Thus the **Red Blood-corpuscles** and the **Skeletal Muscles**, although bathed by fluids which contain a marked excess of sodium over potassium salts, nevertheless, in themselves, contain a very marked excess of potassium over sodium salts. Again, although in fresh-water streams the relative content of potassium is often extremely low, the plants which live in them are capable of storing up a comparatively large amount of potassium in their tissues. One of the most extreme instances of this selection by living tissues of components in disproportion to their abundance in the surrounding medium is that afforded by the presence of **Iodine** in considerable amounts in the tissues of the **Thyroid Gland** in mammals and in the tissues of **Marine Algæ**. Iodine is present in normal blood only in undetectable traces and in sea-water in extraordinarily small amounts.

If we place within a dialyzer an excess of diffusible potassium salts over diffusible sodium salts and dialyze against a solution containing excess of diffusible sodium salts, the proportions of sodium to potassium within and without the dialyzer sooner or later readjust themselves, approaching equality. Now the surface of the living cell, although, perhaps, sparingly permeable to water-soluble substances is nevertheless not absolutely impermeable to them, and in the course of time if the inorganic constituents of the cell are present therein wholly in diffusible forms, the concentrations of the various inorganic components within and without the cell must ultimately attain equality. Even the **One-sided Permeability** of the cell-surface would not alter the *proportions* of the various constituents from those prevailing in the external medium, although their total concentration would, in consequence of this, be constantly maintained at a somewhat higher level than that prevailing in the external medium. Hence this phenomenon admits, as Loeb has pointed out, of only one explanation, namely that the inorganic constituents of a tissue which are found therein in excess of the proportion in which they occur in the fluids which bathe it, must exist within the tissue in the form of non-dissociated and non-diffusible compounds. "If a tissue utilizes one kind of metal in this way, for example K, while another metal, for example Na, is chiefly used for the formation of dissociable compounds with Na as the free ion, the consequence will be that the ashes of the tissue contain K and Na in altogether different proportions from those in which they are contained in the surrounding solution. I think we may take it for granted that, at least, potassium forms a non-dissociable constituent of the protoplasm of a number of tissues of animals and plants" (Loeb.)

The proteins are the only abundant constituents of protoplasm which possesses the amphoteric property necessary for simultaneous combination with acid and basic radicals. We have seen, furthermore, that the compounds of proteins with inorganic bases, acids and salts, do not yield any inorganic ions to the solution; they are non-dissociable compounds in so far as the inorganic component is concerned.

It is to the protein compounds in the main that we must look, therefore, for the origin of the selective ability of tissues.

Many of the **Heavy Metal Salts**, such as those of mercury, silver, lead or copper are highly toxic for living organisms in extraordinarily high dilutions. Even water distilled from a metallic still, or collected in a metallic condenser may be extremely toxic to many forms of life. This phenomenon appeared so impressive to the botanist Nageli that he invented a special phrase "oligodynamic action" to describe it.

The phenomenon is not so surprising as it might appear, however, when we recollect that heavy metal ions are protein precipitants and especially tend to form insoluble and non-dissociated compounds with proteins. The effect of this is to reduce the concentration of heavy metal ions in any region containing protein, and if the protein is surrounded by a medium which still contains free metal ions these will diffuse in to take the place of those precipitated or rendered non-dissociable. These in turn will be removed from the solution and so the process will go on until, although the original concentration of metal ions in the external medium may have been very small, in the end the concentration of combined metal in a cell may be considerably greater and quite sufficient to constitute a lethal dosage. As W. A. Osborne has shown, this sequence of events may be directly observed by placing a protein solution inside a parchment-dialyzer and immersing the dialyzer in an exceedingly dilute solution of mercuric chloride. The mercury quickly attains a higher concentration within the dialyzer than without, because as rapidly as it enters it is bound, and the osmotic gradient remains positive from the medium without to the protein solution within the dialyzer.

THE BIOLOGICAL INDIVIDUALITY OF TISSUES AND TISSUE-FLUIDS.

In discussing the various compounds which the **Proteins** are capable of forming we had occasion in Chapter VIII to dwell upon the existence and the properties of the compounds of proteins with other proteins and especially upon the demonstration afforded by the investigations of Hardy, that the **Serum-globulin** which is separable from blood-serum by dilution and acidification is not present as such in the untreated serum but in the form of a complex, probably arising out of the union of several protein molecules.

The presence of these protein complexes in the tissues and tissue-fluids affords a simple and readily intelligible explanation of what would otherwise constitute an exceedingly puzzling fact, namely, the **Biological Individuality** of the various tissues and tissue-fluids. The individual proteins which are found in the tissue-fluids of tolerably nearly related animals, for example in the tissue-fluids of the various species of *mammalia*, appear, on analysis, to be either identical or

very nearly identical with one another. Thus the casein of human milk has been shown by Abderhalden to be chemically identical with the casein of goat's milk, in so far as the relative yields of the various amino-acids enable us to judge. Similarly the serum-albumins and globulins of goose-blood are identical with those of horse-blood, and the investigations of Abderhalden together with more recent analyses by Gortner and Wuertz have shown that within the closest approximation attainable by present methods of analysis the amino-acid yields from the fibrins of ox-blood, horse-blood, sheeps' blood and the blood of swine, are all identical. Yet when the blood or blood-serum of any species of animal is injected into the circulation of another species it is treated as a foreign intrusion, and results in the appearance of specific "antibodies" such as the **Hemolysins** or the **Precipitins** which react with the blood of the species injected, but with no other. Thus if a rabbit be injected repeatedly with human blood-serum, the serum of this rabbit acquires the abnormal property of causing a precipitate to form when it is mixed with human serum. It makes no difference to the result what human being may furnish the serum, but if we employ sera from other and unrelated mammals we obtain little if any precipitate after mixing with the serum of the immunized rabbit. With the sera of related species some precipitate will be obtained, but it is not so abundant as that which is yielded by human serum. The relationship of man to the primates was thus established upon a quantative basis by Nuttal, to whom the following measurements are due:

Anti-human serum mixed with:

Blood of:	Amount of precipitate.	Percentage.
Man	0.031	100
Chimpanzee	0.040	130[1]
Gorilla	0.021	64
Ourang	0.013	42
Dog	0.001	3
Cat	0.001	3
Tiger	0.0005	2
Ox	0.003	10
Sheep	0.003	10
Guinea-pig	0.000	0
Rabbit	0.000	0
Kangaroo (*Macropus bennetti*)	0.000	0

In a similar manner, if a rabbit be immunized against the serum of some other vertebrate than man, the serum of the rabbit so treated will develop a precipitin for that species and its near relatives, and not for other vertebrates. The blood-serum of each species, and in fact the tissues and tissue-fluids in general of each species are so many separate **Antigens**, producing in immunized animals antibodies which may in certain cases be related to one another but which are clearly not in

[1] The estimate of the quantity of precipitate yielded by chimpanzee-serum was much too high, because, as occasionally happens, the precipitate did not settle properly and its true value could not be estimated.

any case identical with one another. Now as far as our experience extends, all antigenic substances are proteins. All attempts to demonstrate antigenic properties in substances unrelated to proteins have resulted in failure and in particular the investigations of Fitzgerald and Leathes, have shown that the **Lipoids** are non-antigenic. Yet, as we have seen the individual proteins which may be isolated from the tissue-fluids are identical in widely differing species.

If, however, the individual proteins which are separable from blood-serum by chemical procedures are present wholly or in part in the unaltered serum in the form of complexes of several proteins united together, we can readily understand how different sera come to contain differing antigens. Two protein-complexes of this type might well be built up out of identical units, and yet differ fundamentally, owing to differences in the combining proportions, and consequently in the mode of linkage of these units. Just as a wide and conceivably infinite variety of proteins may be built up out of differing permutations and combinations of eighteen or nineteen amino-acids, so an infinite variety of protein complexes might be built up by the union in varying proportions and arrangements of the comparatively limited number of different proteins which are individually separable from a tissue-fluid.

In pursuance of this idea Gay and Robertson and C L. A. Schmidt have investigated the antigenic properties of several compound proteins. If compound proteins differ in their biological specificity from their constituents, then a **Compound Protein** should represent a new **Antigen** giving rise to antibodies for itself, as distinguished from the antibodies for its constituents. Unfortunately a formidable technical difficulty stands in the way of clearly recognizing the presence of antibodies which are specific for the compound protein. This is the difficulty which is constituted by the fact that any protein which is capable of being split by hydrolysis into moieties which are still proteins (in the sense that they are antigenic) gives rise on injection into animals to antibodies not only for itself but also for these split-products. Analogously, a compound protein gives rise to antibodies for its constituent parts, and it is only possible to distinguish between these, which would appear in the blood of immunized animals after injection of the separate constituents, and any antibodies which may be formed for the compound as a whole, in those doubtless exceptional instances in which the antibody for the compound reacts with a constituent which is *not normally antigenic.*

The above-mentioned observers have therefore investigated, from this point of view, certain compound proteins in which one constituent is non-antigenic, such as **Protamine Caseinate**, of which the protamine constituent is non-antigenic and toxic, while the casein constituent is antigenic and non-toxic, and **Globin Caseinate** of which the globin constituent is toxic and non-antigenic.

Protamine caseinate displays no antigenic characteristics which

enable it to be distinguished from casein. It is non-toxic, but whether this lack of toxicity is attributable to the masking of the toxic properties of protamine by its combination with casein, or to the smallness of the proportion of protamine contained in the compound, has not yet been definitely established. It gives rise to antibodies for **Casein** by virtue of its casein-content, just as casein gives rise to antibodies for its infraprotein split-product **Paranuclein**, but it does not give rise to any antibody which will react with its protamine constituent. Globin caseinate, however, differs very markedly in its antigenic behavior from either of its constituents. In the first place it is non-toxic, and the failure to exhibit toxicity can hardly be attributable to dilution of the globin constituent by admixture with casein since globin caseinate contains 66 per cent. of globin (see Chapter VIII). Still more striking is the fact that it yields antibodies which react (*i. e.*, display **Alexin-fixation**) not only with the casein constituent of the compound but also with the globin constituent. It would appear evident, therefore, that injection of globin caseinate into animals gives rise to an antibody which does not appear in response to separate injections of its constituents. We have in this case therefore an instance created in laboratory glassware of what we have assumed to occur in tissue-fluids, namely the formation of a protein complex which differs from other proteins, even from those out of which it is itself built up, in the antibodies to which it gives rise when it is injected into animals.

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PART III.

THE CHEMICAL CORRELATION OF THE TISSUES.

CHAPTER XV.

THE VEHICLES OF CHEMICAL CORRELATION; BLOOD AND LYMPH.

THE COMPOSITION OF THE BLOOD.

The distributing agents which accomplish the transportation of substances from one part of the body to another are the **Blood** and **Lymph.** Through their intermediation oxygen and the products arising from the digestion of the foodstuffs are carried to the tissues, the waste-products which result from their activity are carried from the tissues to the excretory organs, and an exchange of products between diverse and widely separated tissues is also rendered possible. Among this latter class of materials there are included a number of substances which, arising in one tissue or group of tissues, stimulate other and distant tissues to correlated activity. These substances are collectively designated **Hormones,** or chemical messengers (from *ὁρμαω,* I arouse, or excite).

The blood consists of a suspension of cellular elements, the red corpuscles or **Erythrocytes** and the white corpuscles or **Leukocytes** in a pale, straw-colored or almost colorless fluid, the **Plasma.** Of the two types of corpuscles the erythrocytes are much more abundant than the leukocytes, the normal average number of erythrocytes in man lying between five and six million per cubic millimeter of blood, while the leukocytes vary in number between 7000 and 15,000 per cubic millimeter. In other species the number of formed elements per cubic millimeter of the blood may be higher or lower than in man. Thus in the mouse the normal erythrocyte-count lies between ten and twelve million per cubic millimeter.

When the blood is shed from the vessels it forms within a few minutes a gelatinous clot, which is due to the separation from the plasma of an insoluble protein **Fibrin.** On standing, the clot shrinks or undergoes **Syneresis,** expressing a colorless or very pale yellowish fluid, rich in

protein and containing in fact all of the constituents of the plasma with the exception of the formed elements and the protein **Fibrinogen,** from which the fibrin arose. This fluid is termed the **Serum,** and it may be obtained in greater abundance and more rapidly by removing the fibrin from freshly shed blood by whipping it with glass rods or by shaking it up with beads. The fibrin adheres in long strings to the rods or beads and may be removed with them from the fluid which is now termed **Defibrinated Blood.** From this the corpuscles, red and white, may be removed by centrifugalization, the supernatant fluid consisting of serum.

The relative volumes of the plasma and corpuscles may be determined in several ways of which the most accurate is probably the method devised by Hoppe-Seyler, which suffers from the disadvantage, however, of being somewhat lengthy and tedious. Defibrinated blood is employed for the estimation, the removal of fibrin from the whole blood introducing only a very slight error which, if desired, may be separately estimated. Three determinations are made, namely: (*a*) The total protein including hemoglobin in 1000 grams of whole blood. (*b*) The total protein, including hemoglobin, in the blood-corpuscles derived from 1000 grams of blood by centrifugalization followed by repeated washing with isotonic salt solution, until the washings are free from protein. (*c*) The total proteins in 1000 grams of serum free from corpuscles. The difference between (*a*) and (*b*) yields the proteins in the serum contained in 1000 grams of blood, so that the ratio $\frac{a-b}{c}$ yields the proportion of 1000 grams which is constituted by the serum in that weight of whole blood. For example, in an actual estimation, the total protein in a kilogram of blood amounted to 172.9 grams, while the corpuscles from this amount of blood contained 124.0 grams of protein. The serum in a kilogram of blood, therefore, contained $172.9 - 124.0 = 48.9$ grams of protein. One kilogram of serum, however, contained 72.5 grams of protein. Therefore the serum in a kilogram of blood comprised $\frac{48}{72.5}$ ths of a kilogram or 674.5 grams. The **Serum** (or plasma as it is termed before the fibrin is removed from the blood) therefore forms about two-thirds of the whole blood and the corpuscles one-third. This proportion is, however, subject to very wide variations. The blood-count itself, *i. e.*, the number of corpuscles contained in a cubic millimeter of whole blood, is variable and in conditions of **Anemia** may fall to one-half the normal value. Then the volume occupied by the individual corpuscles varies with the osmotic pressure of the serum, hypertonicity involving shrinkage and hypotonicity involving dilation of the corpuscles.

The **Electrical Conductivity** of the whole blood compared with that of the serum derived from it may also be employed, as Stewart has shown, for the determination of the relative volumes of the corpuscles and serum. The results yielded by this and by other methods are in substantial agreement with those furnished by the method of Hoppe-Seyler.

In addition to the red and white blood corpuscles certain other minute formed elements are also found in shed blood, namely the **Blood-platelets.** They are only from one-fifth to one-third of the diameter of the red corpuscles and they do not contain nuclei. There has been very much discussion as to whether they exist in the circulating blood as such, or are not artefacts arising out of the shedding of the blood. They have been regarded by various observers as preformed constituents of the circulating blood, as detritus from the destruction of leukocytes and as protein coagula or spherocrystals, which appear in the blood whenever the endothelium of the bloodvessels is injured. They have, however, been observed by Osler in the blood contained in the freshly excised capillaries of the mesentery, so that injury to the bloodvessels, or shedding of the blood from the vessels is not an essential prerequisite to their formation. On standing in shed blood the platelets swell and finally break up and disappear and there is some indication that those agencies which prevent the disintegration of the platelets also hinder the **Coagulation** of the blood. They appear to consist of protein with a very high admixture of a phospholipin which resembles **Lecithin.**

The **Specific Gravity** of the blood necessarily varies with its total dilution, that is, with the amount of fluid which has recently been absorbed from the intestine. As a rule it remains between the upper and lower limits of 1.060 and 1.054, averaging 1.058 in males and a little less in females. In newborn infants the blood has a higher specific gravity, about 1.066.

The **Chemical Composition of the Blood** is very constant in certain respects and highly variable in others. Thus we have seen that the reaction, osmotic pressure and relative proportions of the various inorganic constituents are exceedingly invariable. The concentrations of proteins, glucose, cholesterol and so forth are, on the contrary, very variable. The following analytical data, cited after Abderhalden, are therefore not to be regarded as affording fixed criteria of the composition of the blood in the different species enumerated, but simply as indications of an approximate average composition. Furthermore the estimations of the inorganic constituents are, as Abderhalden points out, merely of comparative value, since the analytical errors involved in the estimations were high, although presumably of similar magnitude in each of the types of blood investigated.

At the time that the above analyses were made the whole of the **Glucose** in the blood was supposed to be confined to the plasma (or serum). It has since been ascertained by Rona and Masing, however, that the glucose in the blood is contained partly in the erythrocytes and partly in the plasma. It is not, however, distributed between these two elements in proportion to their relative volumes. In addition to the various substances enumerated in these analyses, it must also be remembered that blood contains small amounts of **Amino-acids,** derived by absorption from the intestine, and of waste products such as **Ammonia** and **Urea** derived from the metabolic activities of the tissues.

Substance per 1000 grams.	Pig.		Ox.		Goat.		Sheep.		Horse.		Dog.		Cat.		Rabbit.	
	Corp., 435.09.	Serum, 564.91.	Corp., 325.5.	Serum, 674.5.	Corp., 347.2.	Serum, 652.8.	Corp., 319.2.	Serum, 680.8.	Corp., 397.7.	Serum, 602.3.	Corp., 442.8.	Serum, 577.2.	Corp., 434.0.	Serum, 566.0.	Corp., 372.1.	Serum, 627.9.
Water	272.20	518.36	192.65	616.25	211.35	592.54	200.03	624.16	243.87	551.14	277.71	514.30	270.90	524.17	235.74	518.18
Solids	162.89	46.54	132.85	58.249	135.86	60.25	118.82	56.63	153.84	51.15	165.10	42.89	163.11	41.35	136.37	46.71
Hemoglobin	142.20	...	103.10	...	112.50	...	102.80	...	125.80	...	145.60	...	143.20	...	123.50	
Protein	8.35	38.26	20.89	48.901	18.76	50.96	12.80	46.56	20.05	42.65	2.36	34.05	11.62	33.16	4.55	33.63
Sugar	...	0.684	...	0.708	...	0.822	...	0.708	...	0.90	...	0.74	...	0.860	...	1.036
Cholesterin	0.213	0.231	1.100	0.835	0.601	0.698	1.147	0.891	0.26	0.31	0.56	0.37	0.556	0.339	0.268	0.343
Lecithin	1.504	0.805	1.220	1.129	1.339	1.127	1.329	1.088	1.93	1.05	1.02	0.98	1.354	0.971	1.722	1.105
Fat	...	1.104	...	0.625	...	0.0407	...	0.859	...	0.50	...	0.91	...	0.446	...	0.749
Fatty acids	0.027	0.448	...	...	...	0.398	...	0.4908	0.02	0.36	...	0.70	...	0.282	...	0.507
Phosphoric acid as nuclein	0.0455	0.0123	0.0178	0.0089	0.028	0.0117	0.0235	0.0109	0.05	0.01	0.05	0.01	0.063	0.009	0.040	0.015
Soda	...	2.401	0.7266	2.9084	0.755	2.824	0.760	2.917	...	2.62	1.27	2.39	1.174	2.512	...	2.789
Potash	2.157	0.152	0.2351	0.1719	0.236	0.160	0.236	0.172	1.32	0.15	0.11	0.14	0.112	0.148	1.946	0.162
Iron oxide	0.696	...	0.544	...	0.547	...	0.545	...	0.59	...	0.71	...	0.694	...	0.615	
Lime	...	0.0689	...	0.0805	...	0.078	...	0.089	...	0.07	...	0.06	...	0.062	...	0.072
Magnesia	0.0656	0.0233	0.0056	0.0300	0.014	0.026	0.006	0.027	0.04	0.03	0.03	0.03	0.035	0.024	0.029	0.028
Chlorine	0.642	2.048	0.5901	2.4889	0.514	2.409	0.575	2.516	0.18	2.20	0.60	2.31	0.455	2.360	0.460	2.438
Phosphoric acid	0.8956	0.1114	0.2392	0.1646	0.243	0.154	0.228	0.163	0.98	0.15	0.67	0.14	0.697	0.133	0.835	0.151
Inorganic P_2O_5	0.7194	0.0296	0.1140	0.0571	0.097	0.045	0.088	0.057	0.76	0.05	0.54	0.05	0.515	0.040	0.645	0.040

The **Proteins** of blood-serum consist of an admixture of albumins and globulins. It is quite uncertain how many different proteins the blood-serum (or plasma) may contain, but certain fractions can be readily distinguished from one another. Among the globulins the "**Insoluble Globulin**" or "**Euglobulin**" may be readily separated by simple dilution of the blood-serum with from ten to twenty volumes of distilled water, followed by acidification with dilute acetic acid or with a stream of carbon dioxide. The same fraction separates out on submitting blood-serum to dialysis. An additional globulin fraction, the so-called **Pseudoglobulin** remains in solution, but may be coagulated by half-saturation of the serum with ammonium sulphate, and there are indications that this substance, in turn, is not a single chemical individual. The **Albumin** fraction, which is not coagulable by half saturation with ammonium sulphate, may also not improbably consist of a mixture of proteins. Thus from the serum of the horse, but only with great difficulty from other sera, a **Crystalline Serum Albumin** may be obtained by first removing the globulins by half-saturation with ammonium sulphate and then adding more ammonium sulphate until coagulation of the albumins just begins, and allowing the mixture to stand for some time. Only a portion of the albumin is deposited in crystalline form, however, and we are uncertain whether the portion which does not crystallize merely represents the quantity requisite to saturate the liquid with crystallizable albumin, or whether it represents a different protein.

In addition to the albumins and globulins the blood often contains very small amounts of **Proteose**, and also a glucoprotein, termed **seromucoid** which yields glucosamin on hydrolysis. It is present in blood-serum only to the extent of from 0.2 to 0.9 parts per thousand.

It has been noted by a large number of investigators that the relative proportion of globulins to albumins in the blood-serum may present remarkable abnormalities in persons or animals afflicted with certain **Infections**. Normally the globulins are always less abundant than the albumin fractions, so that the ratio *globulin albumin* is always less than unity. In animals or human beings infected with *Streptococcus* or *Staphylococcus*, however, the ratio may be much more than unity, the globulins in some instances amounting to as much as eighty or ninety per cent. of the total proteins. The question of the origin of this remarkable change is of course one which is of great importance to our understanding of the mechanisms by which the organism protects itself against infections, more particularly since, in the case of **Diphtheria** at least, the **Antitoxins** resulting from infection or immunization have been found to be associated with the globulin-fraction of the serum.

The older analyses aiming at the solution of this problem were subject to very great errors and uncertainties, because of the comparatively large volumes of blood which were required for a single analysis. The proteins were coagulated by alcohol, dried and weighed, while

in another sample the globulins were removed by half-saturation with ammonium sulphate and the resulting solution of albumins was purified by dialysis and its protein content determined by a nitrogen estimation or by coagulating, and weighing the dried coagulum. These processes were tedious, inaccurate and time-consuming, and the large quantity of blood required necessitated restriction of analyses to single samples or to samples taken at rare intervals from the same animals.

The recent **Refractometric Method** of Robertson removes these sources of inaccuracy and permits the determination of the "non-proteins" (including proteoses), globulins, albumins and total proteins in a quantity not exceeding one and one-half cubic centimeters of serum. Samples of this volume may be taken several times in a day from the ear of a rabbit without any evident disturbance due to hemorrhage, and hence the effects of various procedures and administrations may be studied by comparing the composition of the blood-serum of the animals before and at successive intervals after the experimental condition is inaugurated.

The following are average results obtained by the refractometric method with various species of mammals and birds.

Species.	Non-protein, per cent.	Globulin, per cent.	Albumin, per cent.	Total protein, per cent.	Per cent. of total protein.	
					Globulin.	Albumin.
Horse	1.65	3.5	4.0	7.5	47	53
Albino rat	1.61	1.7	4.2	5.9	29	71
Ox	1.34	2.2	5.0	7.2	31	69
Hog	1.49	2.8	4.3	7.1	39	61
Sheep	1.30	1.1	5.2	6.3	18	82
Goat	1.43	1.6	4.9	6.5	24	76
Cat	1.87	2.6	5.1	7.6	34	66
Dog	2.01	1.3	4.8	6.1	21	79
Guinea-pig	1.28	0.9	4.7	5.7	16	84
Hen	1.42	1.1	3.5	4.6	25	75
Duck	2.77	1.9	3.2	5.1	38	62

It must, however, be recollected that the normal proportion of globulin to albumin is subject to considerable fluctuation, not only in different individuals of the same species, but from time to time in the same individual. The following are estimations made by Rowe upon sera derived from eighteen normal persons:

The last four determinations were made upon samples obtained from the same individual on different dates. It will be observed that the globulins in these normal individuals never exceeded thirty-two per cent. of the total proteins, although ranging from this proportion down to sixteen per cent. in different individuals. On the other hand seventeen persons with **Syphilis**, and yielding a strongly positive **Wassermann Reaction** gave values for the proportional globulin content ranging from 26 to 49 per cent., and averaging 35.7 per cent. Eight

persons with **Pneumonia** had a globulin-ratio of from 27 to 50 per cent. and averaging 40 per cent. Other infections showed corresponding increases in the proportion of globulins to total proteins in the serum. Cases of **Nephritis** gave a high proportion of globulin (24 to 50 per cent.) while those in which nephritis was associated with the accumulation of salts and urea in the blood had also, of course, a high non-protein content. On the other hand a series of patients with diabetes gave normal values for the globulin-ratio excepting in one instance in which a local infection was also present. Individuals afflicted with various types of anemia, hyperthyroidism, goiter, hemophilia, chronic bronchitis, pellagra, obesity, lead-poisoning, chronic gastro-intestinal disorders and neurasthenia presented normal values for the protein-ratio, Exceptionally high values of the proportion of globulin to albumin in the blood-serum, therefore, are associated with **Infections** or else with **Toxemias.**

Sample No.	Age.	Non-proteins, per cent.	Total proteins, per cent.	Globulin expressed in per cent. of total protein.
1	27	1.2	7.8	25
2	30	1.3	7.4	30
3	36	1.3	7.3	32
4	21	1.3	7.7	26
5	24	1.1	7.6	16
6	30	1.2	7.4	32
7	32	1.1	8.0	28
8	48	1.2	7.9	27
9	19	1.2	8.2	27
10	25	1.3	7.7	26
11	48	1.2	7.3	30
12	28	1.3	6.8	29
13	23	1.2	7.4	24
14	19	1.25	6.5	29
15	48	1.25	6.7	31
16	25	1.3	7.5	21
17	26	1.3	6.7	25
18	29	1.3	6.8	21
19	26	1.3	7.5	20
20	26	1.3	8.2	21
21	26	1.3	8.2	18
22	26	1.1	7.9	24
Averages	..	1.24	7.5	25.5

The origin of the rise of globulins in infections is still to be sought. It is not due to, or directly correlated with the development of antibodies in the circulation, because as C. L. A. Schmidt has shown, a high degree of immunity to pure proteins may be induced without any rise of globulins. It is not due to the **Leukocytosis** or increase in white blood corpuscles which often accompanies infection, because Hurwitz and Meyer have shown that the leukocyte-count and the globulin increases do not in any degree run parallel to one another, while C. L. A. Schmidt has shown that the leukocyte-count may be reduced to one-half the normal in rabbits by the administration of **Benzole** without causing any significant alteration of the globulin-ratio. It is not due to alterations of bodily temperature, because, as Hanson and McQuarrie

have shown, the previously reported rise of globulins after the administration of **Antipyrin** was due to analytical errors, and does not occur. The same observers have also shown that therapeutic agents which markedly accelerate or retard metabolism, namely **Thyroid Extract** and **Sodium Cacodylate** respectively, are devoid of influence upon the protein quotient, and Hanson has also shown that the previously reported effects of **Starvation** were due to individual fluctuations and that if a sufficient number of analyses be made neither starvation nor heavy feeding is found to affect the quotient in any constant manner. On the other hand Buck has shown that if **Ether** or **Chloroform** be administered for very prolonged periods to animals, so that **Albuminuria** begins to appear, the globulin quotient rises, far more markedly than could be accounted for by an escape of serum-albumin into the urine. This observation may possibly indicate that the true source of the marked alterations in the globulin-quotient which occur in infections and toxemias resides in alterations of the **Permeability** of the tissue-cells. No further evidence bearing upon this possibility is as yet, however, in our possession.

THE COAGULATION OF THE BLOOD.

One of the most remarkable properties of the blood is that which it possesses of clotting or coagulating in a brief period after its issuance from the bloodvessels. The clot which is formed is a markedly contractile one and if it is loosened from the sides of the vessel to which it otherwise adheres, the clot, with its entangled blood-corpuscles, shrinks away toward the center of the vessel, expressing a clear white or pale yellow serum as it recedes. This phenomenon is known as **Syneresis**. If a clot be cut into pieces with a knife or rod, the pieces retract from one another and round up into separate masses.

A number of different agencies are capable of preventing the clotting of blood when it is shed, thus the various **Calcium Precipitants**, such as oxalates, citrates, sulphates and so forth will, if added in sufficient amounts, prevent or delay the coagulation of the blood and in fact a common way of preparing incoagulable blood is to receive the blood directly from the vessels into a solution of sodium or ammonium oxalate. Such **Oxalated Blood** as it is called, remains fluid and incoagulable until, and unless a soluble calcium salt be added to it in sufficient amount to remove all of the calcium-precipitating agent. According to Sabbatini there are minimal and maximal concentrations of **Calcium Chloride** below and above which coagulation is inhibited. The upper limit is a 0.162 molecular solution, the lower about one thousandth part of this concentration. Salts which do not actually precipitate calcium, such as sodium citrate, prevent coagulation by reducing the concentration of free **Calcium Ions** below the necessary minimal limit.

Other agencies which will prevent coagulation are certain solutions

of **Peptones** or **Proteoses.** When these are injected into the circulation, in a very brief period the blood which is drawn from the vessels is found to be incoagulable. The **Peptone-plasma** obtained from this blood by centrifugalization may be induced to coagulate by the mere addition of a suspension of leukocytes obtained from lymph, or by the addition of calcium chloride in excess of the amount already present in the blood, or by acidification with carbon dioxide or acetic acid. Wooldridge has also drawn attention to the very interesting property possessed by some proteins which are probably **Phosphoglobulins,** namely that of inducing **Intravascular Clotting** if injected into the circulation gradually or in small doses; while they render the blood **Incoagulable** if they are injected more quickly or in larger doses. The former effect Wooldridge designated the **Positive Phase** of the action of the protein, the latter he termed the **Negative Phase.** It is an especially remarkable fact that, according to Pickering, albino rabbits, and the Norway hare *when in its albino condition,* are immune from these effects. These various phenomena have not yet received any adequate interpretation.

Another agent which renders blood incoagulable is the extract of leeches' heads, known as **Hirudin.** Certain **Snake Venoms** induce a like effect.

The clotting of the blood is in the first instance due to the transformation of a soluble protein, **Fibrinogen,** into an insoluble modification, **Fibrin.** This was conclusively shown by the investigations of A. Schmidt and of O. Hammarsten. If the plasma obtained from blood be mixed with an equal volume of a saturated solution of **Sodium Chloride** a precipitate or coagulum of fibrinogen is produced which may be washed repeatedly in half-saturated sodium chloride solution, redissolved in dilute sodium chloride, reprecipitated by half-saturation with sodium chloride and again redissolved. This solution of fibrinogen in from 1.0 to 1.5 per cent. sodium chloride will not clot, however long it may be allowed to stand. In order to induce it to clot, another substance must be added to it, to which the name **Thrombin** has been applied.

Thrombin may be obtained from freshly-formed fibrin. It is best prepared from the strings of fibrin which are obtained by whipping freshly-shed blood; these are washed in cold water with constant kneading until all of the **Hemoglobin** has been removed. The fibrin is then squeezed dry, minced with scissors, and then covered with an eight per cent. sodium chloride solution, which does not dissolve the fibrin, but extracts the thrombin which is associated with it. The mixture is placed in a refrigerator for forty-eight hours, and then filtered through cheesecloth. A few drops of the viscous filtrate, added to ten c.c. of the fibrinogen solution, cause immediate clotting, *without the addition of any calcium salt.* On the other hand, thrombin solution unmixed with fibrinogen will not clot, whether calcium salts be added to it or not.

Since calcium is necessary for the coagulation of freshly-shed blood, it might seem reasonable to suppose that the thrombin solution contains combined or associated calcium, which suffices to permit the process to go forward. This is, however, not at all the case, for thrombin may be purified by dialysis and precipitation with **Acetone**, and when this has been done twice the thrombin is found to be perfectly free from calcium.

The true secret of the essentiality of calcium in the clotting of recently shed blood lies in the fact that thrombin, as such, is absent from the circulating blood, and from oxalated plasma. Instead, we have a mother-substance, **Prothrombin** which is converted by calcium salts into thrombin. This fact may be shown in a variety of ways, among which the following may be cited: Wooldridge showed that if peptone plasma be cooled for some time to zero degrees centigrade a precipitate of minute discoidal particles collects at the bottom of the container. They resemble very greatly the **Blood-platelets** and may, indeed, actually be identical with them. When these are removed from the plasma, clotting of the fluid is now very difficult to induce by the customary agents, by carbon dioxide, calcium chloride and so forth. Wright subsequently showed that the same precipitate occurs in oxalated plasma and Hammarsten showed that its removal prevented the subsequent clotting of the plasma by the addition of sufficient calcium chloride to precipitate the oxalate and furnish a favorable excess of calcium ions. If, however, this precipitate be treated with lime salts and the calcium subsequently removed by oxalates, it now is found to contain very active **Thrombin** which quickly induces coagulation in *Oxalated Plasma*. A portion at least of the material in the discoidal particles was, therefore, converted by the calcium salts into thrombin. This constituent is prothrombin.

Another method of preparing prothrombin is that which has been devised by Howell. Oxalated blood is centrifugalized and the plasma is heated to 54° Centigrade. This coagulates the fibrinogen. The filtered plasma is treated with **Acetone**, and the precipitate is collected upon a filter and dried. When the prothrombin is required for use the filter paper is cut into small pieces and extracted for about one hour with dilute sodium bicarbonate solution. This solution does not cause clotting of pure fibrinogen or of oxalated plasma unless it is first treated with calcium chloride (0.2 per cent.).

The circulating blood contains prothrombin, therefore, and it also contains calcium salts, and the question necessarily arises why the prothrombin is not converted into thrombin in the vessels, thus leading to intravascular coagulation? The reason that this does not occur is that the conversion of prothrombin into thrombin requires not only the presence of calcium salts but also another factor, derivable from tissue extracts, which Morawitz termed **Thrombokinase**, but which has recently been identified by Howell as a phospholipin, namely, **Kephalin**.

The prompt clotting which occurs when normal blood is shed is due to something which is added to the blood when it comes into contact with the lacerated tissues over which it flows, or which is derived from the disintegration of the leukocytes or platelets in the shed blood. This can be shown by employing the blood of **Birds** or **Amphibians** in which the white corpuscles do not disintegrate so readily after shedding as they do in the blood of mammalia. If a paraffined cannula be introduced into an artery of a bird and the blood be collected in a paraffined centrifugal tube and directly centrifugalized, the plasma which is obtained either does not clot at all, or only very slowly.

The plasma of birds' blood which is thus obtained may be induced to clot if any of a large variety of tissue extracts, such as leukocyte extract, or extracts of the brain, testes or thymus be added to it. The active substance is soluble in ether and with difficulty soluble in alcohol, and it contains phosphorus and nitrogen. Howell, and McLean have shown that pure **Kephalin**, prepared from brain or other tissues has the same power of inducing coagulation as the whole tissue extract, while other phospholipins, lecithin, cuorin and sphingomyelin are devoid of activity. The activity of kephalin is dependent upon the presence of unsaturated linkages, for hydrogenated kephalin, or kephalin that has become oxidized by exposure to the air, is inactive. That some other factor besides the mere presence of unsaturated linkages determines the action of kephalin is, however, evident from the fact that the great majority of the phospholipins which are devoid of **Thromboplastic Action** also contain unsaturated linkages.

The prothrombin in oxalated birds' plasma is not converted into thrombin by kephalin unless calcium salts are also present. Evidently therefore, both of these factors coöperate in the transformation of prothrombin into thrombin.

Two views of the mode of action of thrombin upon **Fibrinogen** have been advanced: The older view, originally proposed by A. Schmidt, regarded thrombin as an enzyme which converted fibrinogen into fibrin by hydrolysis, just as **Casein** is converted by rennet into **Paracasein**. The foundation of this view was twofold: In the first place the thrombin in the plasma or serum of shed blood is inactivated by heating to 60° centigrade for a few minutes and the majority of the enzymes are similarly inactivated at a like temperature. In the second place very small quantities of thrombin are requisite to produce relatively large quantities of fibrin. Howell, however, has adduced a number of facts which militate against this view. In the first place the apparent thermolability of thrombin is due to the presence of salts or other substances in the plasma or serum, and pure thrombin, freed from inorganic salts by dialysis, will withstand boiling for five minutes or more without total loss of activity. If to the same solution 0.5 to 1 per cent. of sodium chloride be added, boiling for one minute inactivates the thrombin completely. Of course this fact, in itself, does not prove that thrombin is not an enzyme for, as we have seen,

many enzymes, particularly those of bacterial origin, are known which are not inactivated by heating or in which the inactivation is temporary or reversible. Moreover it is not really certain that any *pure* enzymes are thermolabile, since, with one exception, no pure enzymes have ever been prepared. The one exception is that afforded by the **Laccase** or oxidizing enzyme of *Medicago sativa*, which has been shown by Euler to be a mixture of calcium salts of aliphatic hydroxy-acids. A synthetic mixture of calcium glycollate, citrate, malate and mesoxalate has the same oxidizing action as the vegetable enzyme and is unaffected by boiling.

The evidence afforded by the effects of heating is therefore inconclusive either for or against the view that thrombin is an enzyme. Much more decisive is the quantitative relationship of the fibrin-yield to the thrombin which has been added to the fibrinogen solution. The following are estimates obtained by Howell:

0.05	mgm.	of	thrombin	yielded	10.75	mgm.	of fibrin.
0.16	"		"	"	34.00	"	"
0.25	"		"	"	36.80	"	"
0.64	"		"	"	42.50	"	"

Moreover, a submaximal quantity of thrombin acting upon a solution of fibrinogen will never furnish a full yield of fibrin, no matter how much time is permitted for the reaction to take place. Evidently, therefore, thrombin enters into and determines the final equilibrium which is attained and its action cannot be purely catalytic.

The action of thrombin upon fibrinogen is specific in the sense that no other protein is similarly modified by thrombin, but it is indifferent whether the thrombin and the fibrinogen are derived from the same or related or even unrelated species of animals. Thus, Howell has found that the fibrinogen of all vertebrates is converted into fibrin by thrombin derived from pigs' blood.

There remains to consider the part which is played by the various factors which contribute to the formation of thrombin from prothrombin. Reasoning from the analogy afforded by the conversion of **Trypsinogen** into **Trypsin** by the **Enterokinase** of the succus entericus, Morawitz supposed that the conversion of prothrombin into thrombin by tissue-extracts was attributable to an enzyme which he designated **Thrombokinase**. A fact which encouraged this view is that if tissue-extracts be heated to from 56° to 60° Centigrade, they lose their thromboplastic activity, and it was inferred that, like the majority of the enzymes, the thromboplastic substance was thermolabile. Kephalin, however, which is very active in promoting the conversion of prothrombin into thrombin does not lose its thromboplastic powers when it is heated. The solution of this apparent contradiction has been supplied by the investigations of Howell, who has shown that if the coagulum which forms when tissue extracts are heated to 60° be extracted with ether, the dried ether extract has all the thromboplastic

activity of the original unheated fluid. Evidently the kephalin in tissue-extracts is carried down with the protein coagulum, either physically adherent to it or else chemically combined with it.

According to Howell, the activation of prothrombin by kephalin is due to the removal from the plasma of an inhibiting substance, **Antithrombin**, which is present in varying amounts in the blood of different species of animals. The proof for the existence of this substance is as follows: If thrombin in a quantity known to be sufficient to rapidly coagulate a given amount of a solution of fibrinogen be previously incubated for about fifteen minutes at blood-temperature with a small amount of fresh plasma or of plasma freed from fibrinogen by heating to 54° C., the ability of the thrombin to coagulate the fibrinogen is found to have become very much impaired. If, however, the plasma has been previously treated with kephalin, its power of inactivating thrombin is lost or very much weakened. This may be illustrated by the following data, furnished by Howell. The following mixtures were prepared:

Mixture A. Fresh pigs' plasma + equal volume of water.

Mixture B. Fresh pigs' plasma + equal volume of kephalin solution.

The mixtures were allowed to stand for thirty minutes and then heated to 54° C. to coagulate the fibrinogen. The filtrates were then tested for their antithrombin-content as follows:

I

Mixture A, 1 drop + Thrombin 5 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = Partial clot in 65 mins.
Mixture A, 1 drop + Thrombin 4 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = No clot in 2 hours.
Mixture A, 1 drop + Thrombin 3 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = No clot in 2 hours.
Mixture A, 1 drop + Thrombin 2 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = No clot in 2 hours.

II

Mixture B, 1 drop + Thrombin 5 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = Clot in 5 to 10 mins.
Mixture B, 1 drop + Thrombin 4 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = Clot in 5 to 10 mins.
Mixture B, 1 drop + Thrombin 2 drops—Incubation of 15 mins.
+ Fibrinogen 10 drops = Clot in 10 to 15 mins.

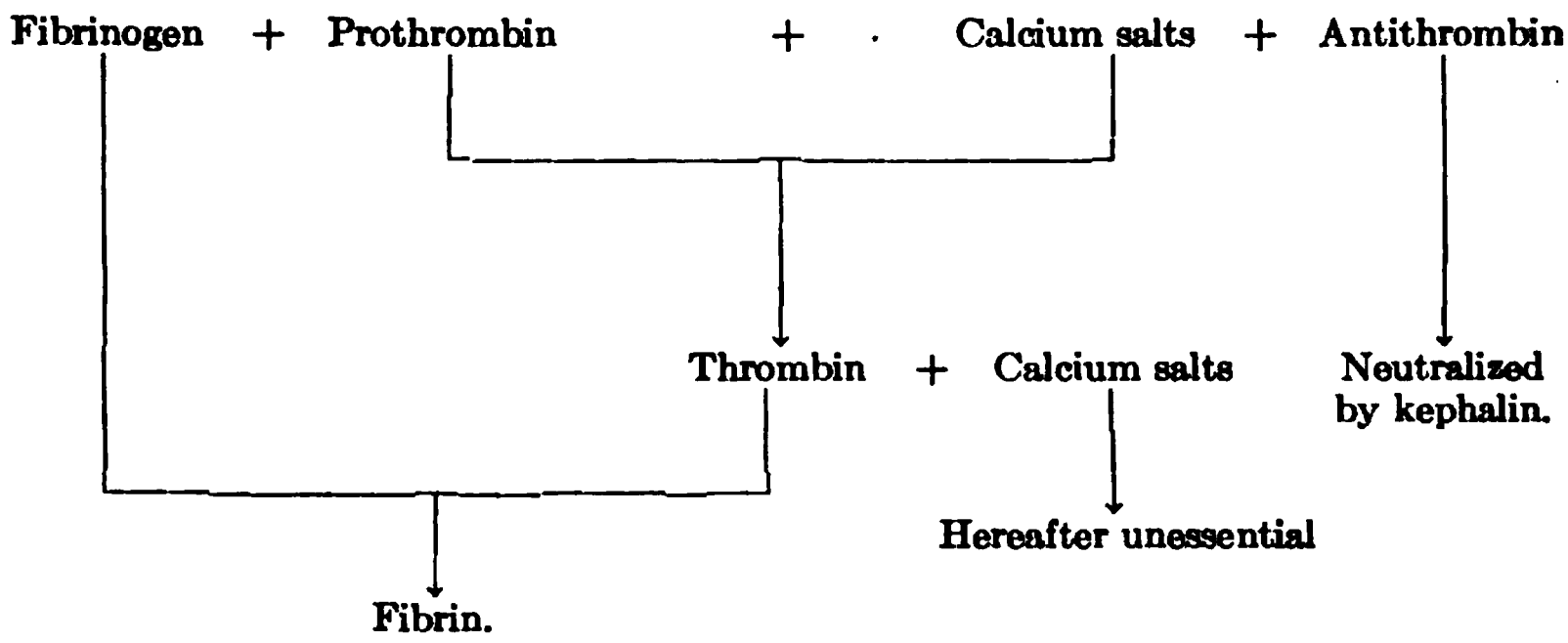
It will be seen that the inhibitive action of mixture A is totally absent in mixture B, which has been incubated with kephalin. These results have found important surgical applications, both for controlling hemorrhages, for which purpose gauze soaked in an aqueous solution (or emulsion) of kephalin is employed, and for the treatment of abnormal tendency to prolonged hemorrhage in cases of **Hemophilia**, which Howell interprets as being due to an abnormal content of antithrombin in the blood of the patients who exhibit it.

Hemophilia is a hereditary condition, and is further peculiar in

that it is almost invariably displayed only by the males of the hemophilic family, while the hereditary tendency to hemophilia is *transmitted* by the females. This peculiar mode of inheritance is also encountered in hereditary **Color-blindness** and in certain other instances of inherited abnormality; it is designated **Sex-linked Inheritance.**

We may therefore sum up the processes and substances concerned in the coagulation of the blood as follows:

The circulating plasma contains:



Howell believes that in addition to antithrombin properly so called, which inhibits the action of thrombin upon fibrinogen, the circulating plasma also contains an **Antiprothrombin** which inhibits the conversion of prothrombin into thrombin by calcium salts and is, like antithrombin, neutralized or inactivated by kephalin.

In regard to the chemical nature of the substances which take part in the coagulation of the blood, **Fibrinogen** is a globulin, being like other globulins coagulable by half-saturation of its solution with ammonium sulphate, but differing from the serum-globulins in being also coagulable by half-saturation of its solutions with sodium chloride. It is not known in what chemical respects **Fibrin** differs from fibrinogen, but the results of Howell and others would seem to render very probable the view that fibrin is a compound of fibrinogen and thrombin. The jelly which is formed by the conversion of fibrinogen into fibrin in the blood or in neutral or faintly acid salt solutions is of exceptional interest because, as Schimmelbusch and Howell have shown, it consists of an interlacing network of acicular crystals enclosing an interstitial fluid (Fig. 19). If, however, fibrinogen be clotted in alkaline solution the jelly, viewed under the microscope or ultra-microscope appears to be structureless. The crystalline jellies display the characteristic tendency of clotted blood to shrink in and express fluid, whereas the structureless jellies do not.

The source of the fibrinogen of the blood appears to be in the **Liver,** since, as Whipple has shown, conditions associated with injury to or insufficiency of the liver, such as **Phosphorus** or **Chloroform** poisoning or hepatic cirrhosis lead to a marked diminution of the fibrinogen content of the blood.

Thrombin may be a protein, but if so then it is protein of unusual properties, for it is not coagulable by heat, and repeated extraction with chloroform appears to remove it from its solution in water. On the other hand it yields the biuret- and Millon reactions and all of the reactions for **Tryptophane**, and it is coagulable by half-saturation with ammonium sulphate. Putrefaction does not destroy it and in fact often seems to increase its activity. These properties appear to indicate that thrombin may be a protein split-product, possibly a proteose.

FIG. 19.—Fibrin crystals viewed under the ultramicroscope. (After Howell.)

The nature of **Antithrombin** is unknown, in plasma it is thermolabile while the antithrombin in leech extracts (**Hirudin**) is not. It is uncertain, however, whether this thermolability may not be due to associated impurities, as it is in the case of thrombin. On the other hand **Antiprothrombin** appears to be a phospholipin, McLean having shown that **Cuorin** from heart-muscle and a phospholipin resembling **Jecorin**

from the liver possess marked ability to inhibit coagulation, the origin of the inhibition being the delaying or prevention of the formation of thrombin from prothrombin by calcium salts.

THE CHEMISTRY OF HEMOGLOBIN.

The red coloring-matter in the erythrocytes of the vertebrates is hemoglobin, a compound protein which is split by hydroylsis into a histone-like protein, **Globin**, and an iron-containing organic acid, **Hematin**. By reason of the power which it possesses of forming a readily dissociable compound with **Oxygen**, hemoglobin accomplishes the transportation of oxygen from the lungs to all the tissues of the body. Other pigments fulfilling a like function are found widely dispersed among invertebrate animals. Thus in the *Arachnidæ Crustacea* and *Mollusca* there is found a protein containing copper, which has been termed hemocyanin and which becomes blue when saturated with oxygen, and colorless when the oxygen is liberated again.

The content of **Iron** in hemoglobin is identical in all species of animals. The following figures, for example, are given by Jaquet:

Hemoglobin from the blood of:	Per cent. of iron.
Dog	0.0336
Horse	0.0335
Ox	0.0336
Hen	0.0335

Assuming that each molecule of hemoglobin contains one atom of iron, this implies a molecular weight for hemoglobin of 16,669 while complete analyses indicate an empirical formula approximating to the following:

$$C_{759}H_{1203}N_{210}S_2FeO_{204}$$

If we examine the **Absorption-spectrum** of well aërated or arterial blood or of a pure solution of hemoglobin which has been shaken with air or oxygen, we find that the transmitted light contains two well-marked absorption-bands, lying between the Fraunhofer lines D and E. The band nearest to D, termed the α band is narrower, but darker and sharper than the β band lying nearer to E. On dilution, the β band is the first to disappear. On concentration the bands become broader and finally appear to coalesce. The center of the α band corresponds to the wave-length $\lambda = 579$, that of the β band to the wave-length $\lambda = 542$. In the **Photographic Spectrum** a band may also be seen in the ultraviolet region, near to G, having its center at the wave-length $\lambda = 415$. This band, which was first detected by Soret, has been proposed as a means of detecting hemoglobin in high dilutions, since it is still perceptible in solutions containing only one part of hemoglobin in 40,000, while the bands in the visible spectrum are no longer perceptible at a dilution of one in fifteen thousand. The absorption-band

in the ultraviolet spectrum is, however, not characteristic of hemoglobin. It is also shown by solutions of its protein component, **Globin**, and more or less distinctly by solutions of many other proteins. It is distinctly visible in the light transmitted through solutions of **Tyrosine**, **Phenylalanine** and other aromatic amino-acids, to which radicals its presence in the protein absorption-spectrum is due.

It was first shown by the English physicist, Stokes, that if blood be placed under a vacuum, or acted upon with a reducing-agent such as an alkaline solution of ferrous sulphate or ferrous tartrate (known as **Stokes' Reagent**), or warm solutions of the alkaline sulphides, the absorption-spectrum of the solution changes. Only *one* band is now to be seen in the visible spectrum, where formerly there were two. This lies between D and E, nearer to D than to E. The same spectrum is supplied by the blood of asphyxiated animals. This absorption-spectrum is due to hemoglobin as distinguished from the **Oxyhemoglobin** which is formed when hemoglobin solutions are aërated. The center of the band lies at wave-length $\lambda = 559$. The band in the photographic spectrum is at the same time shifted, as Gamgee has shown, the center of this band in solutions of **Reduced Hemoglobin** lying nearer the visible spectrum than it does in solutions of oxyhemoglobin. The color of solutions of oxyhemoglobin is the typical scarlet of arterial blood; solutions of reduced hemoglobin are darker, with a slightly purple hue and they also exhibit the phenomenon of **Dichroism**, the color of light reflected from the surface of the solution being green, while transmitted light, as we have stated is red, with a slightly purple tinge.

By the action of oxidizing-agents reduced hemoglobin is rapidly converted into oxyhemoglobin, but the further action of many oxidizing-agents such as ozone, potassium permanganate, potassium ferricyanide and chlorates results in the formation of a modification of oxyhemoglobin which is designated **Methemoglobin**. The absorption spectrum of methemoglobin resembles that of oxyhemoglobin, excepting that the β band is more intense than the α band and a third band is present between C and D. The color of methemoglobin solutions is chocolate-brown, changing to red when the solution is rendered acid, the absorption-spectrum changing at the same time and showing only one absorption-band between C and D. The oxygen-content of methemoglobin appears to be identical with that of oxyhemoglobin, but it is much more firmly combined and is not given up under a vacuum, nor is it displaced by a stream of **Carbon Monoxide**. When, however, methemoglobin is treated with Stokes' reagent reduced hemoglobin is reformed and this in turn forms oxyhemoglobin on shaking the solution up with air. Methemoglobin is often spontaneously formed when arterial blood is allowed to stand in sealed tubes and it may be found in transudates and cystic fluids stained with blood, or in old extravasations of blood following upon injuries.

The blood of animals which have been asphyxiated by illuminating gas is of a peculiar florid cherry-red color, which does not change when

Stokes' reagent is added to it. This is due to the presence of **Carbon Monoxide Hemoglobin**, which may also be obtained by blowing a stream of carbon monoxide or of illuminating gas through a solution of oxyhemoglobin or reduced hemoglobin. In the former case the oxygen combined with the hemoglobin is quantitatively displaced by the carbon monoxide, a given volume of carbon monoxide displacing an equal volume of oxygen. We can readily distinguish between normal arterial blood and the blood obtained after carbon monoxide poisoning, in the first place by the lack of effect of Stokes' reagent upon the color of the carbon monoxide hemoglobin, and in the second place by the effect of adding concentrated sodium hydroxide (specific gravity 1.3) in the proportion of two volumes of sodium hydroxide solution to one volume of blood. Blood containing carbon-monoxide hemoglobin yields a cinnabar-red precipitate, whereas normal blood yields a dingy brown precipitate. Furthermore, **Tannic Acid** yields with normal blood a brownish-green precipitate, and with carbon-monoxide blood a pale crimson-red precipitate. The spectrum shows two absorption-bands similar to those of oxyhemoglobin but nearer to the violet end of the spectrum. The carbon monoxide may be dissociated from the hemoglobin by the prolonged action of a vacuum or of a stream of oxygen or an indifferent gas.

The quantity of oxygen or carbon monoxide which combines with one gram of hemoglobin is 1.34 c.c. at 0° C. and 760 m.m. Hg. This corresponds to one molecule of oxygen or carbon monoxide for every atom of iron in the hemoglobin molecule. If, therefore, we regard the molecule of hemoglobin as containing one atom of iron, the reaction between hemoglobin and oxygen appears as a simple bimolecular reaction as follows:

$$Hb + O_2 \rightleftarrows HbO_2$$

The reaction proceeding from left to right when the partial pressure of oxygen is increased, as it is in the lungs, and from right to left when the partial pressure of oxygen is reduced, as it is in the tissues. Similarly the interaction with carbon monoxide may be represented as follows:

$$Hb + CO \rightleftarrows HbCO$$

Designating the concentration of reduced hemoglobin in any solution by the symbol Cr, that of oxyhemoglobin by the symbol Co, and that of oxygen by the symbol "b," then applying the mass-law to the balanced reaction:

$$\begin{array}{ccccc} Hb & + & O_2 & \rightleftarrows & HbO_2 \\ Cr & & b & & Co \end{array}$$

We would have, at equilibrium:

$$Cr \times b = KCo$$

where "K" is a constant which is characteristic of the equilibrium, and represents the ratio of the velocities of the opposed reactions. The concentration of oxygen in the solution will, of course, be directly proportionate to the partial pressure Po of oxygen in the atmosphere above the solution and to the absorption-coefficient a_t of oxygen in water at the particular temperature "t" which is employed. We therefore have:

$$b = P_o.a_t$$

and:

$$\frac{Co}{Cr} = K.a_t\ P_o$$

Hence in a solution of hemoglobin brought into equilibrium at any given temperature with a mixture of nitrogen and oxygen, such as air, by shaking or by exposure over a very extensive surface, as in the capillaries of the lungs, the ratio of oxyhemoglobin to reduced hemoglobin should be directly proportional to the partial pressure of oxygen in the atmosphere to which it is exposed.

This relationship was investigated by C. Bohr who found so many irregularities which were apparently inconsistent with the equation that he inferred the existence of several different compounds of hemoglobin with oxygen. The whole question was, however, reinvestigated by J. Barcroft and his collaborators with greatly improved technique and it was ascertained that the irregularities observed by Bohr were due to inconstant contamination of the hemoglobin by crystalloids and that in properly dialyzed solutions the relationship deduced from the mass-law holds good with exactitude. The origin of the irregularities in solutions containing inorganic electrolytes resides in the tendency of hemoglobin to polymerize in such solutions, Roaf having found that while hemoglobin in distilled water exerts an **Osmotic Pressure** corresponding to a molecular weight of 16,000, in sodium chloride solution the osmotic pressure corresponds to a molecular weight of 32,000. On rendering this latter solution alkaline the molecular weight of the hemoglobin again falls to 16,000, the weight which is also indicated by the iron- and sulphur-contents, assuming each molecule of hemoglobin to contain one atom of iron.

The influence of **Temperature** upon the **Equilibrium-constant** of a balanced chemical reaction is expressed by the well-known thermodynamical equation:

$$K_T = K_{T_o} \cdot e^{-\frac{q}{2}\left(\frac{T-T_o}{T\,T_o}\right)}$$

where K_T is the value of the equilibrium-constant at the temperature T, K_{To} is the value of the constant at temperature To, "q" is the heat given out by the conversion of one gram-molecule of the substance and "e" is the base of the natural or "Napierian" logarithms. The validity of this equation for the reaction between oxygen and hemo-

globin has also been established by Barcroft, as the following data reveal. The oxygen pressure was constantly maintained at 10 mm. Hg

	Percentage of hemoglobin converted into oxyhemoglobin.				
At	16°	24°	32°	38°	49°
Observed	92	71	37	18	6
Calculated	90	71	41	22	6

from which figures and the above equation it is easy to deduce that "q" or the heat given out when one gram-molecule of hemoglobin unites with oxygen, is 28,000 calories. Now the heat given out when *one gram* of hemoglobin unites with oxygen is 1.85 calories. Hence we have the simple ratios:

$$\frac{\text{weight in grams of one gram-molecule}}{\text{Weight of one gram}} = \frac{\text{Heat given out by one gram-molecule}}{\text{Heat given out by one gram}} = \frac{28{,}000}{1.85}$$

whence the weight in grams of one gram-molecule of hemoglobin is calculated to be 15,200, which estimate, when one recollects the number and variety of measurements which enter into it, is in extraordinarily good accord with the known molecular weight of hemoglobin, namely, about 16,000.

FIG. 20.—Hemin crystals, magnified. (After Preyer.)

By the action of acids, alkalies or heat in the presence of oxygen, hemoglobin can readily be split up with the liberation of **Hematin.** If this hydrolysis is accomplished in the presence of hydrochloric acid the substance obtained is the hydrochloride of hematin or **Hemin,** which may be readily recognized by its characteristic crystalline form (see Fig. 20).

Alkaline solutions of hematin show pronounced **Dichroism,** being red in thick, and green in thin layers, while acid solutions of hematin are brown. The solid substance forms glistening bluish-black amorphous masses. The hydrochloride, however, is brown.

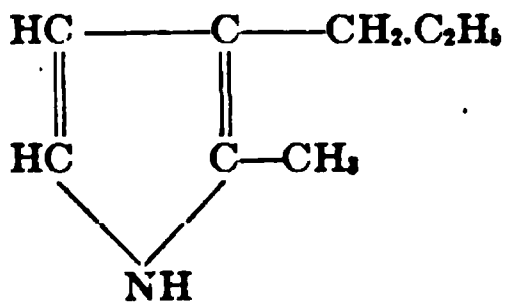
If hematin be dissolved in strong **Sulphuric Acid,** on diluting the

solution a dark red substance, **Hematoporphyrin**, or iron-free hematin is deposited, the iron originally contained in the hematin molecule being left in the solution in the form of **Ferric Sulphate**. Hematoporphyrin is identical with a substance known as **Hematoidin** which is frequently found in the form of microscopical rhombic crystals in old extravasations of blood or apoplectic clots. It is also identical with **Bilirubin**, the red coloring-matter of the bile.

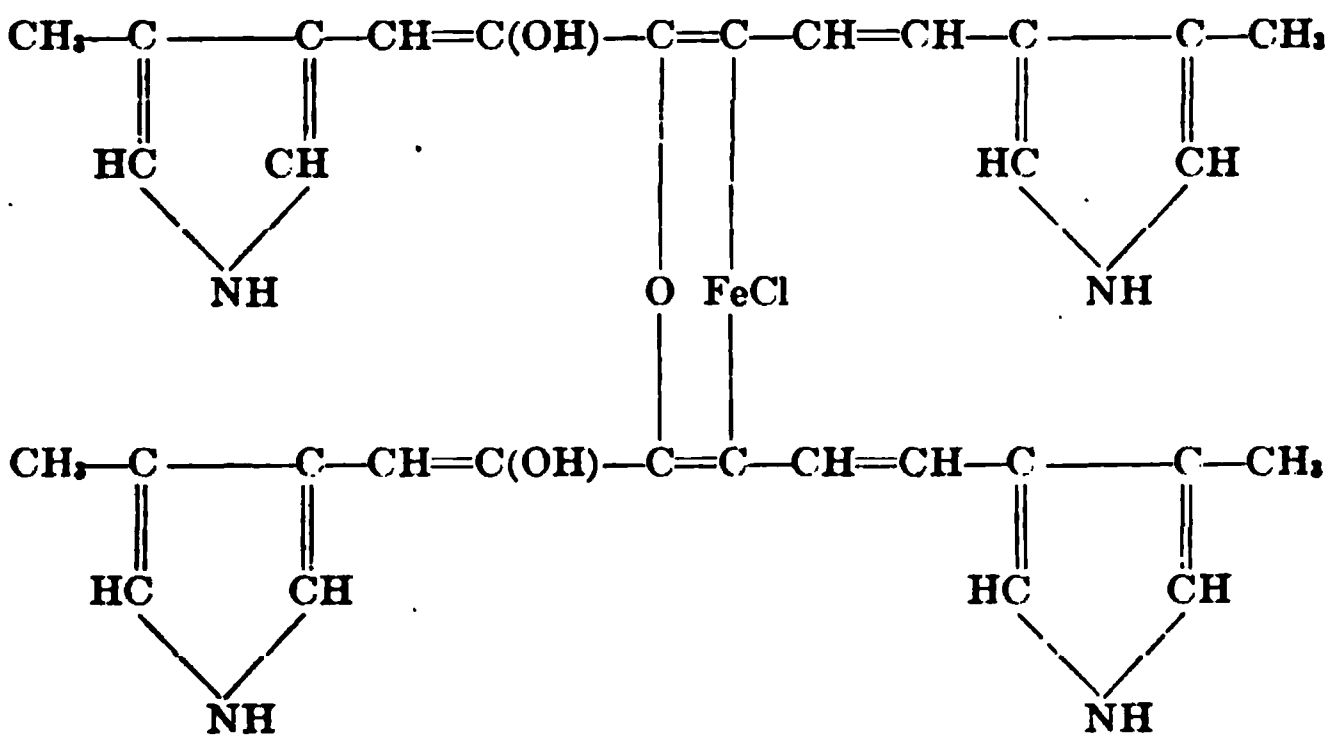
When hemoglobin is decomposed by alkalies *in the absence of oxygen*, we obtain **Hemochromogen**, or "reduced hematin." This substance yields bright red solutions in alkaline media, acids very quickly change it into hematoporphyrin and a ferrous salt:

$$\underset{\text{Hematin.}}{C_{32}H_{32}N_4FeO_4} + 2H_2O + 2HCl = \underset{\text{Hematoporphyrin.}}{2C_{16}H_{18}N_2O_3} + FeCl_2 + H_2$$

By reduction of hematoporphyrin we obtain, among other products, a substance known as **Hemopyrrole**, $C_8H_{13}N$, which is a methyl propyl pyrrole:



From its quantitative composition and the abundance of **Methyl Pyrrole** derivatives among its decomposition-products, it appears probable that hematin may be built up out of four methyl pyrrole radicals united by iron and oxygen. The hydrochloride, or **Hemin** may possibly be represented by the following structural formula:



The extensive investigations of Marchlewski, to whom we owe much of our knowledge of these pigments, have resulted in establishing the very close relationship which exists between hematin and **Chlorophyll**, the green pigment of plants. Thus among the products resulting

from the decomposition of chlorophyll, a substance, **Phyloporphyrin,** is obtained which differs from hematoporphyrin only in containing two hydrogen atoms in the place of two hydroxyl-groups. The attempt has been made to transform hematoporphyrin into phyloporphyrin by reduction, but this attempt has as yet only been partially successful, only one of the hydroxyl groups in hematoporphyrin having been replaced by hydrogen.

The close relationship of hematin to chlorophyll at once suggests the possibility that the necessary radicals for the binding of hemoglobin may be obtained by animals from the decomposition-products of chlorophyll. The pyrrole grouping may of course be obtained from the **Proline** and **Oxyproline** constituents of the protein molecule, but it is a question whether the synthetic activity of the hemopoietic tissue in the red marrow of the bones goes so far as to build up hematin from pyrrole or whether, rather, somewhat more complex fragments of hematin may not be requisite. It is true that chlorophyll is not digestible by the hydrolytic enzymes of our alimentary system, but that does not exclude the possibility of bacterial digestion in the lower intestine, and as a matter of fact, Marchlewski has shown that chlorophyll does actually in part disappear when introduced into the alimentary canal of animals. Abderhalden has suggested that the failure of inorganic-iron therapy in certain cases of **Anemia** may be attributable to lack of certain decomposition-products of chlorophyll in the diet, or to lack of the proper assimilation or utilization of these products which he conceives, may be necessary for the synthesis of hemoglobin.

THE CRYSTALLINE FORMS OF HEMOGLOBIN IN RELATION TO THE BIOLOGICAL INDIVIDUALITY OF THE BLOOD.

The constant percentage of iron in the hemoglobins derived from different **Vertebrata** invites, but does not establish the accuracy of the supposition that the hemoglobins from different sources are identical. While the quantitative composition of hemoglobin must be the same in all species, yet there exist a very large number of conceivable arrangements of the various radicals and groupings in the molecule, and of stereochemical differences not detectable by mere analysis. In fact Reichert and Brown have in recent years very strongly advocated the view that the hemoglobin of every species differs chemically or stereochemically from that of every other, basing their view upon the results of their monumental investigation of the crystalline forms of hemoglobin derived from different sources.

Crystals of hemoglobin are readily obtained from the blood of certain animals by the mere evaporation of blood "laked" by ether. This procedure suffices in the case of the blood of the rat, for example. In many cases it is necessary to cool the blood to zero and in some to add alcohol to reduce the solubility of the hemoglobin. Generally speaking the best method to induce crystallization is to add from one to five

per cent. of **Ammonium Oxalate** to freshly shed blood, which not only prevents clotting but accelerates the process of crystallization, then lake the corpuscles by shaking up the blood with ether, remove the debris of corpuscles by centrifugalization and allow the fluid to evaporate on a microscopic slide. In some cases the nature of the agent employed to lake the blood or induce **Hemolysis** is of importance in determining the ease of crystallization. Thus if dogs' blood be laked with **Toluol**, an abundance of crystals of hemoglobin is easily obtained by merely cooling the laked blood in a refrigerator.

The results of Reichert and Brown have shown that the crystals obtained from the blood of different species are never identical in form. From an enormous number of measurements of crystal-angles, etc., conducted upon hemoglobins derived from a very wide variety of species these observers conclude that the crystals of the different species of any one genus belong to the same crystallographic system and generally to the same crystallographic group, and they have approximately the same axial ratios, or their ratios bear a simple relation to each other. In other words the hemoglobin crystals of any genus are isomorphous, but not identical. In some cases this **Isomorphism** may be extended to include several genera, but this is usually not the case unless, as in the case of the dogs and foxes, for example, the genera are very closely related. On the other hand the oxyhemoglobin obtained from the same species always crystallizes in the same form, although often with a different "habit" when obtained by different methods of preparation. But upon comparing the hemoglobins from different species of a genus it is always found that they differ from one another to a greater or less degree in angles or axial ratio, in optical characters, and particularly in those characters comprised under the general term "**Crystal Habit,**" so that one species can usually be distinguished from another by the form of its hemoglobin crystals (Fig. 21).

A clear relationship is thus seen to subsist between the physicochemical behavior of a constituent of organisms, and their place in the phylogenetic scale of relationships as established by their gross morphology, and a long stride has been taken toward the establishment of a physicochemical basis for morphological distinction. The further, and entirely independent question now arises, however, as to the *chemical origin* of the observed physicochemical phenomena.

Our experience with the crystallography of inorganic and the simpler organic substances has led us to infer with a considerable degree of confidence that substances which show differences in crystallographic structure are different chemical substances. Crystal form is affected even by isomeric modifications which analysis, unaided by other methods of investigations, fails to reveal. Now the enormous number of atoms in a protein molecule encourages, at first sight, the supposition that an enormous and indeed, for all practical purposes, an infinite number of isomerides are possible between which the most refined methods of analysis would not enable us to distinguish, but

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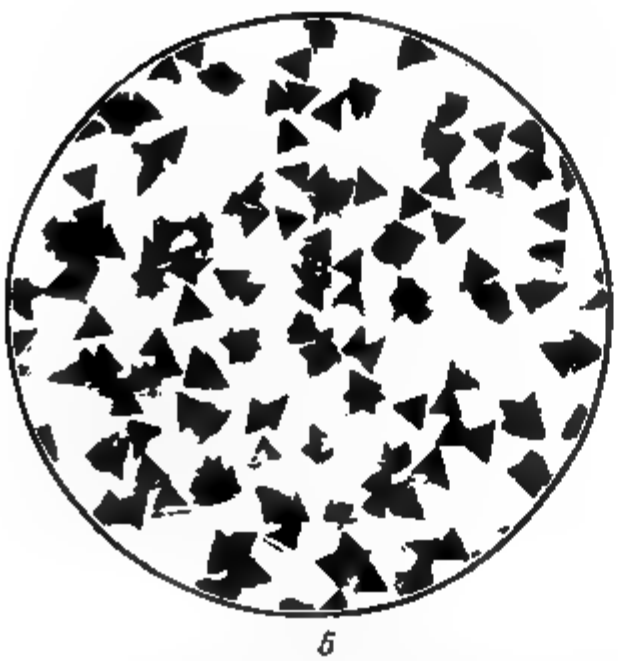

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FIG. 21.—Oxyhemoglobin crystals of various animals. *1*, the goose; *2*, the Tasmanian devil (*Sarcophilus ursinus*); *3*, the kangaroo (*Macropus giganteus*); *4*, the horse; *5*, the guinea-pig, *6*, the long-armed baboon (*Papio langlædi*). (After Reichert and Brown.)

which would very probably differ from one another in the morphology of their crystals. In point of fact, however, the available number of isomers would be very greatly restricted by the necessity of maintaining unaffected the amino-acid groupings of the protein moiety, which could not differ materially in different species without leading to decided differences in the chemical behavior of the hemoglobins, which have not been observed by any investigator. Further doubt is thrown upon this interpretation of the facts by the observation of Hüfner, recently confirmed with the utmost precision by Butterfield, Heubner and Rosenberg, and Schumm, that the characteristic **Absorption-bands**, and the ratio of the absorption of light in different parts of the spectrum of hemoglobin are absolutely identical in species so far removed from one another as the horse and man (Schumm) or the rabbit, sheep, and hog (Heubner and Rosenberg). Now these are properties which we would anticipate might be materially affected by internal differences of atomic arrangement.

Further reason for doubting the correctness of referring the differences of crystal structure displayed by the hemoglobins of different animals to internal differences in the molecule of the hemoglobins is supplied by the observation of Loeb and Brown that the *crystal-form of the hemoglobin of the mule is intermediate in character between that of the horse and that of the donkey*. For if we assume that each different crystal-form represents a different internal atomic arrangement of the hemoglobin molecule, then the number of such arrangements, even if very great, must nevertheless be limited. The number of possible forms of crystals must, therefore, also be limited, and, moreover, the possible modifications of forms must be discontinuous, *i. e.*, there must exist forms between which no intermediate forms are possible. This being the case it would be very remarkable indeed were the hybridization of two closely related species to lead to the synthesis of a new isomeric variety of hemoglobin not yet appropriated by any existing species of animal and, in addition, lying *between* the hemoglobins of the parent-species. If analogous phenomena should be displayed by all hybrids and by all varieties and mutations that might have arisen or might conceivably arise in the future, we would have to admit that the hemoglobins already recognizable as differing from one another in crystalline form are only a small proportion of those which are realisable.

A much more reasonable supposition is that embodied in the view that the differences in crystal-form observed by Reichert and Brown were attributable, not to the internal variation of atomic grouping in the hemoglobin molecules, but to external variations in the milieu from which they are crystallized. The technique adopted by Reichert and Brown was to induce crystallization directly in the laked blood. Now we know from the observations of the immunologists that the blood-plasma from any species of animal differs antigenically from that derived from any other species, and since all known antigens are proteins, we infer that the proteins or, more probably, the compound **Protein Complexes** in blood-plasmas derived from different species are in

certain definite respects different from each other. The crystals of each species studied by Reichert and Brown were therefore deposited from a different medium, and it is not improbable that the observed differences between the crystals are attributable to these known differences in the media in which they were formed. It is well known that crystal-habit is modified by alterations of the medium from which the crystals are deposited. That modifications of this origin, so great as to prevent inclusion of the crystals formed in different media in the same isomorphic series, have not hitherto been observed in the domain of inorganic chemistry is not improbably attributable to the simpler character of the conditions accompanying crystallization in inorganic or non-colloidal media. We have seen that there are many reasons for supposing that proteins, even in solution, are disposed in a certain reticular structure (cf. Chapter XIII), and if, as the facts which we dwelt upon in connection with the properties of the compounds of proteins with each other would seem to indicate, characteristic protein complexes, formed by the union in differing proportions of a relatively small number of simpler protein components, exist in each type of blood-plasma, we may well suppose that the reticular structure of the solutions comprising these plasma would likewise differ from one another. Having regard to the markedly cohesive properties of proteins, crystallization within the meshes of such a reticulum might very conceivably, through external strains imposed by points of attachment to the reticulum, modify the effects of the internal strains which find their expression in crystal form.

This hypothesis finds decided support in the fact, first observed by Halliburton, and confirmed by Reichert, that the crystal form of oxyhemoglobin derived from a given species may be profoundly modified by admixture with the blood of another species. The following are illustrative results obtained by Halliburton, the "normal" form of rat-hemoglobin crystals being rhombic, those obtained from guinea-pigs being normally tetragonal, and those from squirrels' blood hexagonal.

Blood of	Mixed with that of	Form of hemoglobin crystals deposited from the mixture.
Rat	Squirrel	Both rhombic prisms and hexagons present.
Rat	Guinea-pig	No rhombic prisms of the shape usually seen in rats' blood present; no tetrahedra; crystals are all rhombic prisms with hexagonal habit.
Squirrel	Guinea-pig	Hexagonal plates and retrahedra both present; many tetrahedta imperfect; the tetrahedra all reduced to about half the size of those prepared from the unmixed blood of the same guinea-pigs.
Dog	Squirrel	Fine rhombic needles and hexagonal plates both present in abundance.
Dog	Guinea-pig	The greater number of the crystals formed are very small tetrahedra about a quarter the size of those prepared from the blood of the same guinea-pig. The optical properties are, however, the same; rhombic prisms, very slender, like those of dogs' blood are also seen.

According to Reichert, the degree of modification of crystal form induced by admixture of two bloods depends very greatly upon the proportion in which they are mixed.

In view of these facts there can be little doubt that the nature of the milieu in which crystallization occurs does play an important part in determining the form of the crystals which are deposited, and having regard to the known individuality of the plasma from different biological species, it would appear unnecessary to seek further for the origin of the differences in crystal form of the oxyhemoglobins derived from blood of different species of animals.

In this way we can also interpret the changes in crystal-form which Halliburton observed to result from repeated **Recrystallization** of hemoglobin, for as Wichmann and more recently Katz have shown, the crystalline proteins swell in, or absorb the surrounding fluid menstruum in a manner analogous to the swelling of jellies. A number of recrystallizations are therefore required to remove completely traces of the original menstruum in which crystallization occurred.

Bradley and Sansum believe that the hemoglobins from different animals are antigenically different, because guinea-pigs sensitized to ox- or dog-hemoglobin failed to display **Anaphylactic Shock**, or reacted but slightly to hemoglobins of other origins, while they reacted strongly to the hemoglobin with which they were sensitized. As the hemoglobin preparations employed by Bradley and Sansum were admittedly (with the exception, they believe, of dog-hemoglobin) not free from contamination by serum, the interpretation of these results is open to serious question. Doubt is especially thrown upon this evidence for the specificity of hemoglobins from different species by the fact that the animals sensitized to the purest preparation of hemoglobin employed, that of the dog, reacted strongly, not only to dog-hemoglobin, *but also to dog-serum*. Observers are not all agreed that pure hemoglobin is antigenic; its protein component, globin, certainly is not, and having regard to the investigations of Wichmann and Kat, cited above, revealing the marked ability of crystalline proteins to absorb the menstruum from which they are deposited, and to the observation of Schulz and Zsigmondy that **Egg-albumin** must be recrystallized from 3 to 6 times in order to remove appreciable contamination by other proteins, we may infer that in all probability the specificities demonstrated by Bradley and Sansum are serum-specificities and not hemoglobin-specificities.

THE CHEMICAL DETECTION OF BLOOD.

The chemical detection of blood and identification of blood-stains is often of the very gravest medicolegal import. The older methods of detection depended upon microscopical identification of blood-corpuscles, and, of course, a very slight degree of putrefactive change, or the drying of a blood-stain upon a garment rendered the detection

of these formed elements impossible. This was succeeded by the far more delicate and reliable **Hemin Test**, which consists in placing a drop of suspected fluid or saline extract of shreds of stained fabrics, upon a microscope-slide, adding a crystal of salt and a drop of glacial acid, heating the fluid to boiling by passing the slide to and fro over a small flame, and then examining the fluid, as it cools, for hemin crystals. This test may be successfully employed with samples of blood far advanced in decomposition. A still more delicate test, however, is the **Benzidine** reaction. This depends upon the power of an enzyme or **Peroxidase**,[1] which is present in blood, to decompose **Hydrogen Peroxide**, liberating nascent oxygen which oxidizes the benzidine with the production of a green or blue color. Properly conducted, this test will detect one part of blood in three hundred thousand, which means, in effect, that a murderer may wash his blood-stained hands in a bath full of water, and yet if any drainage remains unemptied at the bottom of the bath, the fact that he has done so may be detected with certainty. Nevertheless even more delicate tests are available. Thus Buckmaster has found that if an alcoholic solution of **Guaiaconic Acid** be added to blood together with hydrogen peroxide, a blue color may be produced at a dilution of one in five million. This test is also given by perfectly fresh **Milk** collected and bottled with aseptic precautions, but it is not given by the milk which is ordinarily obtainable in the market.

For the identification of the *Species* from which blood is derived we rely upon the antigenic **Specificity** of blood. The suspected fluid is mixed with anti-human serum prepared by immunizing a rabbit against human blood. The mixture is incubated, and the occurrence of a flocculent precipitate indicates that the suspected fluid contained either human blood or the blood of an anthropoid ape. Since "The Murders in the Rue Morgue" must be admitted to have constituted an entirely exceptional problem, the alternative thus presented does not furnish any serious basis for uncertainty.

THE ORIGIN AND COMPOSITION OF LYMPH.

The tissues are not, excepting in a very few situations, bathed by blood itself, but by the **Lymph**, which is derived from blood, and through the intermediation of which the substances dissolved or combined in blood are brought into physical contact with the protoplasm of the living cells.

There was formerly much discussion of the question whether lymph is elaborated from the blood by a process of active secretion, constituting an **Exudate**, or whether, on the contrary, it is a **Transudate**, derived from the blood by passive filtration. Heidenhain believed it to be an exudate for the following reasons:

[1] It is considered probable that hemoglobin itself is the agent which brings about this decomposition. Catalase, which is also present in blood, decomposes hydrogen peroxide with the production of inactive, or molecular oxygen.

If the lymph were derived from the blood by mere leakage or filtration through the walls of the bloodvessels, the rate of leakage should be greater, the greater the pressure of the blood. The rate of flow of lymph in the **Thoracic Duct**, however, does not always decrease when the arterial blood-pressure decreases, nor does it always increase when the arterial pressure increases. Then, again, the injection of strong salt solutions into the circulation might be expected to withdraw fluid from the lymph-spaces by osmotic attraction, yet the lymph-flow from the thoracic duct is actually increased by this procedure. Finally certain specific substances, particularly crayfish extract, and extracts of leeches or shell-fish, certain **Proteoses** and also the South American arrowhead poison **Curare** cause a very great increase in the flow of lymph, as Heidenhain supposed, by stimulating the secretory activity of the vessel-walls through which the lymph issues into the interstices of the tissues.

Nevertheless Starling has conclusively demonstrated that the production of lymph is, after all, a process of passive filtration. The phenomena adduced by Heidenhain, convincing as at first sight they appear to be, are nevertheless simply attributable to the fact that the **Permeability** of the bloodvessels for lymph varies very greatly in different parts of the body. These differences in permeability lead to differences in the rate of filtration of lymph no less pronounced than the difference in the rate of filtration of water through paper and through unglazed porcelain. The most permeable vessels are the capillaries in the **Liver**, while the capillaries in the skeletal muscles are almost impermeable. We can render the capillaries in the leg-muscles permeable by heating them to 56° C., and in this way cause such extensive transudation of lymph that a frog's leg, so treated, becomes rapidly edematous. If the blood-pressure in the liver be raised or lowered the lymph-flow is raised or lowered in like proportion, but the pressure in the liver and that in the general arterial system do not always run parallel, so that the departures from parallelism between arterial pressure and lymph-flow observed by Heidenhain were not inconsistent with the view that lymph is a transudate, mainly furnished by the vessels of the liver. Strong salt or sugar solutions simply alter the distribution of the interstitial fluids, causing a general imbibition of fluid into the vascular system, and a **Hydremic Plethora** which results in readjustment by more rapid filtration into the lymph-spaces in the liver. If we previously withdraw from the vascular system enough blood to equal the volume of fluid which is attracted into it by the subsequent injection of salt or sugar, no plethora results, and no increased flow of lymph ensues.

The various **Lymphagogues** or lymph-producing substances alluded to above cause an increased transudation by the injury they cause to the walls of the blood-vessels, greatly increasing their **Permeability**, and producing an effect analogous to that of heating to 56° C.

The composition of lymph is very variable. In general it may be

regarded as resembling blood-plasma, but containing a larger proportion of tissue waste-products and of fatty substances derived from the chylous lymph-vessels of the intestine.

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CHAPTER XVI.

EXAMPLES OF CHEMICAL CORRELATION.

THE CHEMICAL CORRELATION OF RESPIRATORY ACTIVITIES.

The normal **Respiratory Movements** of the diaphragm and intercostal muscles are adjusted to the average need for oxygen which is imposed by the normal functional activities of our tissues. The performance of function and the maintenance of the temperature of the body necessitate an expenditure of energy which, since the **Hydrolyses** which occur in living tissues are usually but slightly exothermic, must be derived for the greater part from energy liberated by **Oxidations.** As the tissues which are primarily concerned in the performance of mechanical work are the muscular tissues, variations of their activity may most clearly be seen to necessitate corresponding variations in the rapidity and extent of the oxidations upon which their power of performing work depends. Of all the various tissues of the body, in fact, the muscles are subject to the most sudden and extreme variations of functional activity, being at the one moment in the state of moderate tension which is the normal condition of rest, and at the next expending all the energy required, for example, to lift the whole weight of the body up a steep incline. To provide a sufficient oxygen supply to render possible at all times, without alterations of the respiratory rhythm, the maximal expenditure of energy by the skeletal muscles, would require a very great wastage of energy by the respiratory muscles themselves, or else the relegation of an excessive proportion of the bodily volume to performance of respiratory functions. The mechanism actually and normally employed provides an amplitude of oxygen for customary and moderate needs and when the oxygen requirements of the skeletal muscles renders the customary means of ventilating the body insufficient, then the efficiency of ventilation is temporarily enhanced by a very decided increase in the frequency and amplitude of the respiratory movements.

Now there is no immediate or obvious connection between the movements of the respiratory muscles and those of the skeletal muscles. There is no anatomical or mechanical connection or association between them that would render it *a priori* probable that the motions of the one group of muscles would tend to synchronize in frequency and extent with those of the other. Moreover, the respiratory movements in the adult higher vertebrates are known to be primarily under the control of a particular region of the **Medulla Oblongata,** situated in floor of the fourth ventricle, and designated the **Respiratory Center.**

Stimulation of this area enhances the rate and amplitude of the respiratory movements. Its narcotization or injury depresses or annuls the respiratory movements. The actual synchrony is therefore not directly between the skeletal muscles and the respiratory muscles, but between the skeletal muscles and the nervous tissues of the respiratory center. Here we have an even less obvious relationship between tissues which nevertheless act in perfection of harmony, and the source of this harmony lies in a chemical and not in a spatial or mechanical interdependence of the tissues which participate in it.

The initial effect of deprivation of oxygen or of interference by mechanical or other means with the entrance of air into the lungs is an increased amplitude and frequency of the respiratory movements, a condition which is designated **Hyperpnea**. This is succeeded by the stage of **Dyspnea**, in which the still more rapid movements become almost convulsive in character, until finally every muscle which can directly or indirectly assist in the effort to fill or empty the lungs is brought into intense activity. This activity is quite uncontrollable, as the reader may convince himself by the simple endeavor to "hold the breath" for a prolonged period. If, finally, the lack of oxygen, or obstruction to the passage of air, still defeats the object of these exertions, a relatively sudden cessation of respiratory convulsions sets in, due to paralysis of the respiratory center, and the animal or man is now said to have suffered **Asphyxia**. If, on the contrary, instead of deprivation of oxygen or obstruction to the intake or exit of air, we have an exceptionally efficient ventilation of the lung, by forcible and repeated inflation or by a series of rapid and very deep voluntary breathing movements, then a condition of temporary suspension of the activity of the respiratory center sets in, a condition known as **Apnea**, which is purposely cultivated by divers and swimmers before undertaking a period of prolonged immersion below the surface of water. Either no desire to breathe is experienced for a perceptible interval, or the desire is very easily controlled by a voluntary effort.

After a somewhat longer lapse of time than usual, however, the desire to breathe is again acutely felt, and the respiratory movements thereafter become again uncontrollable by any effort of the will.

Now the effects of suspended breathing are twofold. In the first place the supply of oxygen to the blood, and therefore to the tissues, is cut off and the available oxygen in the body, free or combined in easily dissociable compounds like **Oxyhemoglobin** is soon exhausted by the irreducible minimum of oxidative change which accompanies the life of all the tissues. In the second place the carbon dioxide which ultimately results from these oxidations cannot escape from the body, and therefore accumulates in the blood and in the tissues.

The stimulated activity of the respiratory center which accompanies inadequacy of respiration is due to some change in the blood which irrigates it. This is conclusively shown by the fact that if the cerebral circulations of two animals be "crossed," so that the blood from the

carotid artery of the one animal supplies the brain of the other, then the prevention of effective respiration in the animal of which the brain is receiving normal blood produces no hyperpnea or dyspnea, while the other animal, which can breathe freely, but whose brain is supplied with blood from an asphyxiated animal, shows every sign of respiratory distress.

Two possibilities evidently exist, therefore. Either the stimulation of the respiratory center which results from prevention of the normal ventilation of the lung is due to a lack of sufficient oxygen in the blood which supplies the respiratory center, or else it is attributable to the accumulation of carbon dioxide in the blood-supply. It may be that both of these factors play some part in determining the total result,[1] but by far the predominant part is that which is played by the accumulation of **Carbon Dioxide**, as will be clear from the following considerations:

In the first place it has long been known that a very slight increase, relatively speaking, in the carbon-dioxide content of the inspired air leads to a considerable acceleration and increase in amplitude of the respiratory movements. To bring about a like increase, by a mere decrease of oxygen, provided thorough ventilation of the lungs be secured by unobstructed breathing movements, requires a very much greater diminution of oxygen pressure than the requisite increase of carbon-dioxide pressure. Then, again, in the performance of muscular work there is little or no deficiency of oxygen in the blood, but the content of carbon dioxide must be increased, for the output of carbon dioxide in the lungs is increased and, furthermore, the carbon-dioxide content of the air contained in the alveoli of the lungs, the **Alveolar Air**, is increased by work. The performance of work being in fact accomplished by means of the liberation of energy derived from oxidations, the end-products of these oxidations, among which carbon dioxide and water are predominant, must accumulate in the tissues during the performance of work, and therefore be more abundantly contained in the blood than during rest. The supply of oxygen to the tissues, on the other hand, is normally superabundant, and the muscular tissues, moreover, contain a certain reserve-store of oxygen, and when they are excised from the body, they can contract and perform work for a considerable period in an atmosphere which is devoid of oxygen. In fact, the saturation of the arterial **Hemoglobin** with oxygen is so nearly complete in normal respiration that the hyperpnea which results from energetic exercise would be devoid of utility if its object were the introduction of more oxygen to the tissues. Finally, we have seen that **Apnea** may result from enhanced ventilation of the lungs, but this is not due to an increased intake of oxygen or of saturation of the tissues therewith, for it may be brought about as well by

[1] It is improbable that lack of oxygen is in itself a stimulus to the respiratory center or any other tissue. The apparent stimulation, if it occurs at all, is only indirectly attributable to deficiency of oxygen.

repeated and forced filling and emptying of the lungs by an indifferent gas, such as hydrogen or nitrogen, and when this has been done, it is found that the carbon-dioxide tension, in the alveolar air and therefore in the arterial blood, is decidedly lower than normal.

The increased carbon-dioxide tension of the blood in obstructed breathing is therefore the stimulus which excites the activity of the respiratory center. But the carbon dioxide may conceivably act in either of two ways upon the center, namely as a specific chemical stimulant, or else indirectly by the increase in the hydrogen ion concentration of the blood which it brings about.

The experiments of Winterstein were designed to elucidate this question. This investigator introduced acids into the blood which was passing by the carotid artery to the brain, and he obtained a decided acceleration of the respiratory rhythm as a result. Prior to these experiments of Winterstein, it had also been shown that in frogs in which the floor of the fourth ventricle has been exposed, the direct application of acids to this area of the medulla causes acceleration of the respiratory rhythm, while that of alkalies slows it. Both experiments were inconclusive, however, because they did not enable us to ascertain whether the acids administered excited the center by virtue of the hydrogen ions which they contributed to the blood, or by setting free carbon dioxide from bicarbonates and thus increasing the carbon-dioxide tension of the blood. Subsequent experiments by Laqueur and Verzár threw more light upon the question, tending to show that carbon dioxide is a specific stimulant for the respiratory center, for they found, using Winterstein's technique, that the nature of the acid added to the cerebral circulation profoundly affected the result, and the efficiency of the various acids did not run parallel to their "strength" or dissociation into ions. Carbon dioxide, lactic acid and various fatty acids are much more efficient stimulators of respiration than the strong mineral acids. Evidently, therefore, we have here to deal with an effect which is not wholly a hydrogen-ion effect, but also in part an effect involving the undissociated molecule or the anions of the acid employed.

THE CHEMICAL REGULATION OF THE CIRCULATORY SYSTEM.

Removal of the **Suprarenal Glands** in animals, or their destruction by disease (usually tubercular) in man, is followed by the rapid appearance of intense prostration and muscular weakness. The blood-pressure falls to an extremely low level, and death finally supervenes. In man, the destruction of the glands by disease is usually somewhat gradual and the symptoms are correspondingly slow to develop. They are of the same description as those which develop in animals when these glands are excised, but, in addition, a peculiar patchy bronze-like pigmentation of the skin occurs. The nature of the pigment which is deposited in these patches is unknown, but it is highly probable that

it is chemically related to **Adrenaline**, for adrenaline, like the **Tyrosine** from which it is probably derived, is readily converted into highly colored substances by oxidizing-agents and by oxidizing-ferments, especially by the **Tyrosinase** which occurs in many vegetable tissues, particularly those of fungi, and also in certain animal tissues, as, for example, in tumors arising in the suprarenal bodies (melanomas) and in the ink-sac of the cephalopod *sepia*.

The blood-pressure raising substance, adrenaline, which occurs in the medulla, or inner portion of the suprarenal gland, is capable, when administered intravenously, of correcting the excessively low blood-pressure in animals with the suprarenals excised, or in Addison's disease, but it does not avail to prevent the ultimate death of the animals and it is probable that other substances essential to life are produced by these glands besides adrenaline. It is possible that the cortex of the gland, which has an epithelial origin and differs both in structure and embryological development from the medulla, may play an equally essential part in the bodily economy. This is indicated in the first place by the fact that serious symptoms of adrenal insufficiency may accompany degenerative changes affecting the cortex alone, and furthermore by the remarkable effect of extensive superficial **Burns** upon the cortex. Burns or scalds, if at all extensive, are followed by lesions of the suprarenal cortex and especially by minute hemorrhages therein. These changes are progressive for several days following the injury, and are prominent in instances of deferred death resulting from extensive burns or scalds. The unusual abundance of **Lipoids** and especially of **Cholesterol Esters** in the suprarenal cortex is suggestive of a function related to the lipoid metabolism, but the nature of this function remains unknown.

It is of the active physiological principle of the medulla, namely **Adrenaline**, that our knowledge is most extensive. This substance, when injected intravenously in minute amounts (0.001 mg. and upward in a dog), causes a marked rise in blood-pressure (Fig. 22). This phenomenon is one of many consequences of the general action of adrenaline in stimulating the **Myoneural Junctions** of the muscles innervated by the sympathetic system. The action is not upon the nerves themselves, or upon their anatomically visible endings, for it is more and not less pronounced when the nerve is cut and allowed to degenerate up to and including its anatomical connection with the muscle. The action is not upon muscle-fibers themselves, for in the first place muscles not innervated from the sympathetic system are not affected by adrenaline, and in the second place the muscles innervated by the sympathetic system are not all affected alike, for if inhibitory fibers predominate the muscle is relaxed, while if stimulatory fibers predominate the muscle is contracted. The glandular tissues are variously affected, for if the adrenaline stimulates their secretory activity, the contraction of the bloodvessels and the consequently diminished blood-supply operate in a contrary direction. In the kidneys the diminished

blood-supply at first reduces the output of urine, but when the blood-pressure effect passes off, which it does rather rapidly, a decided **Diuresis** follows.

Local subcutaneous administration of adrenaline so constricts the adjacent vessels that its absorption is thereby much delayed and its action is prolonged. It is upon this fact that the extensive employment of adrenaline in minor surgical operations depends. Bleeding is prevented, and an unobstructed view of the tissues is secured for the period of the operation. Furthermore, local **Anesthetics** simultaneously applied share in the difficulty of absorption, and therefore continue their local analgesic action for a longer period than would otherwise be attainable. The tendency to post-operative hemorrhage is, however, said to be enhanced by adrenaline and it is also to be remembered that

FIG. 22.—Blood-pressure (*B.P.*) and bowel volume (*I.V.*) of cat. At *A* injection of adrenaline. The blood-pressure rises and bowel volume diminishes, indicating constriction of the mesenteric vessels. As these relax again the blood-pressure falls. The vagi had been divided previously, so that there is no secondary slowing of the heart. (After Cushny.)

the normal defense of the tissues against infections is supplied by the blood and by the leukocytes which the blood and lymph contain, so that a measure of natural protection against bacterial invasions is denied the tissues by this procedure.

An important affect of intravenous injections of adrenaline is the appearance of **Glucohemia** and its resultant, **Glycosuria.** The power of the liver to polymerize glucose is apparently rendered deficient and the normal equilibrium between glycogen and glucose in the liver-tissues is shifted in favor of the glucose.

It has been established in many ways that minute quantities of adrenaline are constantly present in the blood. That this must be continually supplied to the blood by the suprarenal glands follows from the fact that injected adrenaline very rapidly disappears from the circulation and the tissues, being apparently destroyed or, at all events,

converted into substances devoid of the typical activities of adrenaline. It is, however, a question that is still being debated whether or not the small amounts normally present in the blood-stream actually influence the tone of the vascular system, and help to maintain the normal blood-pressure. The extremely low blood-pressure in Addison's disease, however, and the marked effect of adrenaline in raising it, stated even to be more marked than in normal individuals, would seem to point rather decisively to a constant relationship between the functional activity of the suprarenals and the maintenance of normal blood-pressure.

According to Cannon, however, one of the most important functions of the suprarenals is to assemble a group of conditions appropriate for the defense of the organism in an emergency. Violent **Emotional States**, such as fear, rage or pain (and also anesthesia) lead to a marked discharge of adrenaline from the suprarenal glands, and to all the effects which arise from intravenous injection of adrenaline. The intravenous injection of adrenaline in the cat will, as a matter of fact, elicit very many of the most easily recognizable external signs of fear without the application of any other stimulus. Thus the hair of the back and tail is raised, and the pupils of the eyes are widely dilated. It is to the presence of an excess of adrenaline in the blood that the glycosuria of violent emotions, the so-called **Emotional Glycosuria**, is partly due. It has been shown by Macleod that stimulation of the splanchnic nerves, which innervate the suprarenal glands, results in the production of glucohemia which is partially attributable, however, to the direct stimulation of the liver through the hepatic branches of the splanchnics.

The effect of emotional stimulation, operating through the splanchnics, is to increase the adrenaline in the blood and thereby to increase the blood-pressure, quicken the heart-beat and thus enhance the mobility of the blood and the rate of access and exit of the raw materials and products of metabolic activities. The liability to external hemorrhages is reduced owing to the constriction of peripheral vessels, and also to a definite reduction of the coagulation-time of the blood, which is another result of adrenaline administration. The instantly available nutritive materials for the muscle-cells are increased by the mobilization of sugar-reserves. In short the animal is placed in the best attainable condition for a sudden extreme effort and the sustainment of possible injury. In conflicts, or in efforts to escape from more powerful predatory forms, the suprarenal glands probably constitute an essential factor in success or failure.

THE CHEMICAL CORRELATION OF THE PROCESSES OF DIGESTION.

The arrival of foodstuffs in the stomach is preceded by a considerable secretion of **Gastric Juice**, and, in consequence, the processes of gastric digestion are enabled to go forward without delay. The correlation

between the acts involved in the intake of foodstuffs and the secretory activity of the glands of the gastric mucosa is, however, as the classical researches of Pawlow have shown, nervous in origin, and not chemical, arising in part from reflexes arising from optical and olfactory stimuli and in part from gustatory and tactile impressions. The detailed consideration of their mechanism belongs therefore to the domain of physiology rather than to that of biochemistry. The subsequent steps in the process of digestion involve, however, a very remarkable series of chemical correlations.

During gastric digestion the pyloric sphincter remains closed, and it opens to permit the discharge of the stomach contents only when the digestion of the proteins has attained the stage of nearly complete conversion into **Proteoses** or **Peptones**. The mechanism which regulates the tone of the sphincter is nervous, but the stimulus which releases the reflex dilatation is chemical, and consists of the presence in the lower end of the stomach of foodstuffs containing a definite excess of hydrogen ions. This is very clearly shown by the investigations of Cannon, who found that the period which elapses before the first opening of the sphincter and discharge of **Chyme** into the intestine, is proportional to the quantity of substances in the food which are capable of neutralizing acids. Thus, solutions of sugar or starches are retained for but a brief time in the stomach, but the period of their retention may be enhanced very greatly by admixture with substances which neutralize free acids. Meat and other dietary constituents which contain proteins on the contrary are retained for a relatively prolonged period.

When the acid chyme has been discharged into the duodenum in sufficient quantity to induce a certain acidity of the contents of the upper part of the small intestine, the pyloric sphincter again closes in accordance with the general law governing the musculature of the intestine, namely, that any localized stimulus causes relaxation below and contraction above the stimulated point.

When the **Chyme** is being discharged from the stomach through the dilated pyloric sphincter, an augmented outflow of **Pancreatic Juice** is already travelling down the pancreatic duct to meet it. The time-relations of the production of the two digestive fluids, gastric juice and pancreatic juice, is illustrated by the following data obtained by Pawlow.

Time after partaking of food.	Gastric secretion after 100 grams of meat.	Pancreatic secretion after 600 c.c. of milk.
1 hour	11.2	8.8
2	8.2	7.5
3	4.0	22.5
4	1.9	9.0
5	0.1	2.0

the maximal secretion of pancreatic juice coinciding, in time with the moment when the maximal quantity of chyme is leaving the stomach.

The immediate origin of this phenomenon resides in the acidity of the gastric contents which, upon the opening of the pylorus, come in contact with the mucosa of the upper part of the duodenum, and, in fact, a copious secretion of pancreatic juice may be elicited by simply bathing the duodenum with dilute acids, for example 0.4 per cent. hydrochloric acid. The same result is obtained if the acid be introduced into the jejunum, but not when it is introduced into the ileum. The exciting agent, however, is not the acid itself, for the injection of 0.4 per cent. hydrochloric acid (one-tenth normal) into the circulation is without effect upon the secretion of pancreatic juice. The excitation of the pancreas is, on the other hand, not accomplished through a nervous reflex because it occurs, and is undiminished when the portion of the intestine which is treated with acid is isolated from all nervous connections, and furthermore, it continues after the administration of **Atropine**, which paralyzes the endings of the secretomotor nerves.

The actual intermediary which brings about this correlation is a substance **Prosecretin** which is present in the mucous membrane of the duodenum and the jejunum, and which is changed by acids into **Secretin**, a diffusible, water-soluble, heat-resistant substance, which has the property of specifically stimulating the secretory cells of the pancreas. If the mucous membrane be scraped from the surface of the duodenum and rubbed up in physiological saline solution (0.9 per cent. NaCl) the filtered extract which is thus obtained may be injected into the circulation without eliciting any secretion of pancreatic juice. If, however, the extract be previously boiled, or acidified and then neutralized, the injection will now be followed by a copious secretion of pancreatic juice. In normal digestion the transformation of the prosecretin in the duodenal mucosa into secretin is accomplished by the acid chyme, and the secretin which is formed is carried by the blood-stream to the cells of the pancreas.

Secretin occurs in the mucosa of the intestine in all vertebrates and even in the intestines of fishes. It is diffusible, is not destroyed by boiling, and is soluble in acidified solutions of mercuric chloride, being precipitated on neutralization. It appears to be a nitrogenous base, and is probably an amine derived by **Decarboxylization** from an amino-acid or from an amino-acid derivative. Acidified extracts of the intestinal mucosa and of many other tissues, contain **β-Iminazolyl Ethylamine** but this substance is devoid of action upon the secreting cells of the pancreas. The chemical identity of secretin has therefore not been established. A nitrogenous base having a similar action upon the pancreas is known, however, namely **Pilocarpine**, a trimethyl ammonium derivative obtained from the leaves of *Pilocarpus jaborandi*.

It must be stated, however, that acids are not the only substances which will bring about a secretion of pancreatic juice when they come into contact with the duodenal mucosa. **Fats** are particularly active in causing secretion of the pancreatic juice after their entry into the duodenum, probably, however, only after they have been partially

converted into soaps. The origin of this effect is unknown. The **Soaps**, like other **Calcium Precipitants** are strong stimulators of nerve fibers and nerve endings, and the contention of Pawlow, that their action upon pancreatic secretion arises reflexly through stimulation of nerve endings in the intestine, is therefore not unfounded. On the other hand it has been suggested that the soaps formed from fats in the intestine, convert prosecretin into secretin or into some substance of like action, which is carried to the pancreas by the blood-stream. Other substances causing an especially abundant flow of pancreatic juice are **Chloral Hydrate** and **Ethyl Alcohol.**

The chemical coördination of the processes of digestion does not end, however, with the coördination of the secretory activities of the digestive glands. If care be taken to excise the pancreas without allowing the tissues to come into contact with the mucous membranes of the intestine, or if the secretin is collected by means of a cannula placed in the duct, so that it is obtained before it touches the intestinal surface, it is found that the fluid is devoid of proteolytic activity. Yet the moment after it arrives within the intestine a very intense proteolytic activity is developed. The reason for this is that **Trypsin** is not present within the tissues or secretions of the pancreas as such, but in the form of a proteolytically inactive precursor which is designated **Trypsinogen.** The conversion of trypsinogen into trypsin will not occur spontaneously, under aseptic conditions, even after a period of weeks or months. If, however, the fluid is momentarily acidified and then neutralized, the conversion of trypsinogen into trypsin is found to have been completed within the brief period of exposure to the action of hydrogen ions. A more prolonged exposure results in partial or complete destruction of the trypsin, and since the rate of secondary destruction of the enzyme is proportional to the dissociation or "strength" of the free acid, it is safer to employ, for the conversion of the trypsinogen, a weakly dissociated acid, such as **Salicylic Acid,** which furnishes a sufficiency of hydrogen ions to activate the trypsinogen but decomposes the active trypsin relatively slowly.

In actual digestion the activation of the trypsinogen may be brought about in part, it is true, by the admixture of the pancreatic juice with the acid chyme, for the contents of the duodenum are acid to **Litmus,** although alkaline to **Methyl Orange,** throughout the greater part of its length. But that another chemical mechanism exists in the intestine which is capable of bringing about very rapid and complete activation of trypsinogen is shown by the fact that when the pancreatic secretion is poured into the empty intestine, the trypsinogen which it contains is found to have been activated within a very brief period after its arrival within the intestine; in fact mere contact with the surface of the intestinal mucosa for a few moments suffices to bring about a considerable degree of activation, and under such circumstances, of course, the reaction of the fluid remains consistently alkaline.

This activation is brought about by a substance which is contained

in the secretions of the intestinal glands comprising the so-called **Succus Entericus.** The activating constituent is designated **Enterokinase,** and because of the fact that it is destroyed or inactivated by heating, and is furthermore active in very small quantities, it has been generally assumed to be an enzyme, and in fact Pawlow has termed it a "ferment of ferments." Nevertheless the proof that enterokinase is an enzyme is very imperfect. Many substances are modified by heat which are not enzymes, of course, and the small amount of the material required to activate a large volume of pancreatic juice may merely be expressive of the minute quantity of trypsin which is actually present in the secretion of the pancreas. We have no method of quantitatively estimating trypsinogen and enterokinase except in terms of each other and we have no data which could enable us to arrive at an estimate of the actual weight of trypsinogen which is activated by a given weight of enterokinase. More conclusive evidence of the enzymatic character of enterokinase would be afforded if we were to find that a limited quantity of succus enterícus will activate very large quantities of pancreatic juice provided, only, that sufficient lapse of time be allowed for the completion of the process. But from the results of Hamburger, Hekma and others, it appears that the contrary is actually the case, and that there is a quantitative relationship between the amount of succus entericus which is added to pancreatic juice and the amount of trypsin which is produced.

We have stated that the pancreatic juice, as produced by the secretory cells of the pancreas, is proteolytically inactive. While this is generally the case, it is not necessarily or invariably so. The juice obtained by the action of **Secretin** is, it is true, invariably inactive, but juice obtained by stimulation of the secretomotor fibers in the vagus usually contains active trypsin, and juice containing preactivated trypsin may also be obtained after the administration of certain foodstuffs, particularly diets containing a high proportion of meat. The seat and mechanism of this activation is unknown.

In general, however, it is evident that the proteolytic powers of pancreatic juice must be much enhanced by admixture with succus entericus, in part through the activation of trypsinogen by enterokinase and in part owing to the fact that succus entericus contains **Erepsin,** an enzyme capable of splitting peptones or casein, but not other proteins, to amino-acids. The digestion of protein by mixed proteolytic enzymes is always more rapid and complete than when a single enzyme is present, because different enzymes attack different linkages preferentially so that in the presence of two or more enzymes a larger number of amino-acid linkages are rendered susceptible to rapid disruption. This being the case it is a fact of interest and importance that pancreatic juice itself, according to Pawlow, stimulates, by its presence, the secretion of succus entericus. At all events during gastric digestion the secretion of fluid from the glands of the intestine is very small, but after passage of the chyme into the intestine and the coinci-

dent inflow of pancreatic juice and bile, the secretion of succus entericus is greatly increased. According to some observers, however, this increase is attributable to secretin, which is believed by them to stimulate the intestinal glands as it does the glandular cells of the pancreas. It is difficult at present to disentangle these alternative possibilities, and further investigation is evidently required before the relative parts played by secretin and the pancreatic juice itself in promoting the secretion of succus entericus can be correctly evaluated.

THE CHEMICAL CORRELATION OF THE ORGANS OF GENERATION.

The **secondary sexual characters** of the male, such as the growth of the beard and the deepening of the voice in man, the development of horns in the ram and of the comb and tail-feathers of the cock, have long been known to be attributable to the development of the **Testes.** Castration has long been practised both in man and in animals for the purpose of preventing the development of secondary sexual characters, and of bringing about the psychic and metabolic modifications which also accompany the excision of these organs. The removal of the testes in man before the onset of puberty prevents the appearance of the beard and the deepening of the voice which characterises that period of development, and hardening of the epiphyses of the bones is delayed, so that the legs and arms grow to an unusual length in proportion to the size of the whole body. In certain varieties of sheep only the males are possessed of horns, and in these varieties castration of the young male altogether suppresses the development of the horns. Similarly the castration of cocks suppresses the development of the comb. If, however, the excised testicle be implanted in another part of the body, as, for example, in the peritoneal cavity, then the secondary sexual characters develop normally, the penis grows to its normal dimensions, the seminal vesicles and the prostate develop as if the testes were actually functioning as a generative organ, and yet, not only are the testes prevented by lack of communication with the **Vas Deferens** from discharging spermatozoa but, as a matter of fact, the spermatogenic tissues of the testes dwindle away, and the production of spermatozoa actually ceases. The effect of this organ upon the development of the secondary sexual characters is therefore, evidently, not attributable to its spermatogenic tissues, and appears to be due to the **Interstitial Cells** which are normally present between the seminal tubules and become increased in number in the transplanted organ. Since these tissues are provided with no duct for the conduction of their products to the exterior, the channel of transmission of the substances from the interstitial cells, or, as Steinach calls them collectively "The puberty-gland," to the tissues which they affect, can only be the general circulating media, the blood and lymph. The puberty-gland is, in fact, an example of the **Ductless Glands**, or **Endocrine Organs.**

In the female, the excision of the ovaries leads to a more or less pronounced tendency toward the acquirement of masculine characteristics. Very marked effects upon the male, however, are elicited if the ovary be transplanted into the tissues of a castrated animal of the same species. In this case not only do the secondary sexual characters of the male fail to develop, but those of the female take their place, even to the development of the **Mammary Glands**. Here, again, the effect appears to be attributable rather to the interstitial elements of the ovary, than to the reproductive elements.

A remarkable instance of the converse effect, namely, suppression of female characteristics by secretions from the male organs of generation, is supplied by the sterility which is almost the invariable rule in the females of heterosexual twins in cattle. A female of this type is known to cattle-breeders as a *Free-Martin*. It has been ascertained by F. R. Lillie that in cattle a twin pregnancy is almost always a result of the fertilization of an ovum from each ovary and development begins separately in each horn of the uterus. The ova, in the course of development, however, meet and fuse, and the bloodvessels from each side anastomose in the connecting part of the chorion, so that each embryo receives part of its blood-supply from the other. Both the arterial and venous circulations overlap, so that a constant interchange of blood takes place. If both are males or both are females no harm results; but if one is a male and the other female, the reproductive system of the female is largely suppressed in its development, and certain male organs even develop in the female. The effect of this is to render the female incapable of reproduction.

A recurrent cycle of changes occurs in the **Ovary** of the adult female which results in the intermittent discharge of mature egg-cells from the ovarian tissues into the Fallopian tubes leading into the cavity of the uterus. The ovarian tissues contain a number of vesicles, lined with epithelium and each containing an ovum, which migrate toward the surface of the ovary, at the same time increasing in size. These are the **Graafian Follicles**, which periodically rupture, discharging the ova which they contain. The discharge of the egg into the Fallopian tubes may or may not coincide with the period of menstruation, in fact such evidence as we possess tends to show that the two processes, while coinciding approximately in frequency, do not occur with strict synchrony. The ruptured Graafian follicle, after the discharge of the ovum, undergoes a series of degenerative changes which culminate in the formation of the **Corpora Lutea**, which when mature appear as spherical masses of yellowish cells, disposed in a more or less columnar manner, the columns of cells radiating from the center.

The **Menstrual Fluid** in man consists of blood and shreds of cast-off uterine epithelium, diluted by the secretions of the mucous glands of the uterus. It contains a very high percentage of **Calcium** and for this reason Blair Bell has suggested that it may be related phylogenetically to the egg-shell of birds or of a remote common ancestor of the birds

and mammals. However this may be, a considerable storage of calcium occurs in the tissues of the female prior to menstruation, and this excess of calcium is suddenly discharged during the period of menstruation. Having regard to the immense importance of the precise value of the $\frac{Na}{Ca}$ ratio in determining the susceptibility of nervous tissues to stimuli, it appears not unlikely that some of the nervous accompaniments of menstruation, and particularly the hyperirritability of the uterus which leads to the phenomenon of painful menstruation or **Dysmenorrhea** may be attributable in part to the sudden reduction of the calcium-content of the tissues which occurs at this period.

The menstrual blood usually does not clot at all, or if it clots it does so very slowly. This remains the case even if fibrinogen be added to it, and, as we have seen, calcium is not lacking. It can hardly be deficient in kephalin, or thrombokinase, since the fluid contains so much material arising from the breaking down of the tissues lining the cavity of the uterus. It appears likely, therefore, that the mucous secretions of the uterus contain a substance similar to **Antithrombin** or hirudin in its action upon the coagulation of blood.

When the fertilized egg becomes imbedded in the wall of the uterus a proliferation of the uterine wall results in the outgrowth of a **Placenta** which subsequently provides the developing embryo with circulating blood derived from the mother. We have here a remarkably exact coincidence of events and we are led to inquire why the tissues of the uterus are aroused to the production of this outgrowth at the very moment when it is about to be required?

An answer to this question has been afforded by the very important discoveries of L. Loeb. This observer has found that in the female guinea-pig, for a period of some ten days following the phenomenon of **Ovulation**, any injury to the uterine wall results in the outgrowth of a placenta. The injury may be of the nature of a slight incision, in which case a localized growth occurs which may be duplicated at other points in the uterus, so that as many as twenty different placentæ may be formed in this way in a single uterus. Or the injury may consist of the irritation afforded by the presence of a foreign object, such as a thin glass rod or a number of particles of paraffin. In this case the growth of placental tissue may become so great as to interfere with the nutrition of the newly formed tissue and induce its degeneration and autolysis. The formation of placentæ is prevented if the ovaries are extirpated or even if the **Corpora Lutea** which they contain are excised. The stimulus which arouses this reaction of the uterus to mechanical irritation comes, therefore, from the corpora lutea. If the corpora lutea are not excised at once, and placentæ are permitted to form, they attain a smaller size and degenerate more rapidly if the ovaries or the corpora lutea are excised before their full development is attained.

Among the many correlations which underlie and render possible the development of the embryo, the next into which we have attained some measure of insight is that which obtains between the development

of the embryo and the development of the **Mammary Glands** of the mother. As the fetus grows the mammary glands of the pregnant female hypertrophy until a portion of the hypertrophic tissue begins to break down and give rise to a secretion of milk, and this stage of development is attained at the moment when the fetus is approaching the full term of gestation, and is about to be delivered.

It has been ascertained that this remarkably exact synchrony of the development of such widely separated organized bodies as the fetus and the mammary glands of the mother is brought about by the circulation in the blood of some as yet unindentified substance which is elaborated by the tissues of the **Placenta.** If a saline extract of the placentæ of rabbits be injected repeatedly into the circulation of virgin rabbits, the mammary glands hypertrophy just as they would if the animal were pregnant, and finally secrete milk which may be expressed from the nipples. Another factor, however, which may possibly contribute to the development of the mammary glands and their secretion of milk is the slight measure of hypertrophy of the **Pituitary Gland** which invariably accompanies pregnancy. The boiled aqueous extract of the posterior lobe of the pituitary gland contains a nitrogenous base of unknown constitution, designated **Pituitrin,** which increases the irritability of the muscular walls of the uterus, causes an increase in the volume of the urine and stimulates the secretion of milk, the latter effect being a very unusual one for any pharmacological agent to bring about. The large and repeated dosages of placental extract which Starling and Lane-Claypon found to be necessary to bring about the degree of hypertrophy of the mammary glands which is requisite for the production of milk may possibly have been attributable to the absence of the assistance, in these experiments, which is afforded in actual pregnancy by the enhanced activities of the pituitary body.

That the hyperdevelopment of the mammary glands of the mother is due to the presence of stimulators circulating in the blood, and not to any reflex nervous stimulation of the glandular tissues, is shown, not only by the above-cited experiments, but also by the fact that the effect of these substances is not confined to the mother, but extends to the embryo, which is not connected by any nervous channels with the tissues of the mother. It is a familiar fact that the breasts of newborn infants frequently secrete a few drops of milk or may be made to do so by brief manipulations of the nipples. The milk thus obtained was known in former days as "witches' milk" and was accredited by the lady practioners of a hundred years ago with many important properties of a supernatural description.

When the development of the embryo has reached a certain stage, **Uterine Contractions** bring about the expulsion of the fetus. We have here another example of curiously exact coincidence in time. It is not a question of the size of the developing fetus ultimately bringing about such a degree of distention of the uterus as to induce a special tendency to contraction, for even the same individual may deliver infants in

successive births of very varying size in proportion to the bodily dimensions of the mother. The moment of delivery is, in fact, primarily determined by physiological factors in the mother, rather than by the stage of development of the fetus at term. This may be very clearly seen by comparing the **Variability** of the duration of gestation with the variability of the weights of the infants which are delivered.

The ordinary method of measuring the variability of any quantity which is adopted by statisticians consists in expressing it in terms of the percentage ratio of the **Standard Deviation** of the quantity measured to its average value. The standard deviation is the square root of the mean square of the observed deviations from the average. Thus, consider the following illustrative sets of measurements.

1	11	101
2	12	102
3	13	103
4	14	104
5	15	105

It is obvious at a glance that the figures in the first column are very variable, those in the second column moderately so, and those in the third volumn relatively invariable or approximately constant. When we wish to express this impression in arithmetical terms we proceed as follows:

Average of the first column.	Average of the second column.	Average of the third column.
3	13	103

the deviations from the average are in each case 2, 1, 0, 1 and 2. The sum of the squares of these deviations is $4+1+0+1+4=10$. The mean square is therefore 2 and its square-root, which is the standard deviation, is 1.414. The variability of each of the columns of figures is the ratio of this quantity to the average, expressed as a percentage, which works out as follows:

Variability of first column.	Variability of second column.	Variability of third column.
47.1 per cent.	10.9 per cent.	1.37 per cent.

Our impression of the relative variability of the three columns of figures is thus expressed in quantitative terms, the actual meaning of the results being, that in the first set of figures two-thirds[1] of the recorded values will be found to differ by less than 47.1 per cent. from the mean, in the second set two-thirds of the recorded values will differ from the mean by less than 10.9 per cent. of its value, and in the third set of recorded values two-thirds will fall within 1.37 per cent. of the mean.

Applying this method to the study of the comparative variabilities of the period of gestation and of the weights of the infants delivered thereafter, we find that the two variabilities bear no proportion to

[1] Or, more precisely, 68.27 per cent.

one another, for while the variability of the weight of newborn infants is 14 per cent., that of the length of the period of gestation is only 4 per cent. There can be little influence exerted by the size of the fetus upon the length of gestation, therefore, for otherwise the variability of the period of gestation would be nearly as great as the variability of the size of the infants delivered. It is evident that heavy infants are carried *in utero* for a longer period and light infants for a shorter period than would correspond to their relative development.

We must therefore look to maternal rather than to fetal events for the source of the determination of the period of gestation. Now the investigation of the physiological condition of the mother yields indications of two factors which, as the term of pregnancy approaches, must enhance the muscular irritability of the uterus. The first is the hypertrophy of the **Pituitary Gland**, to which reference has been made above. The aqueous extract of the posterior lobe exerts a very marked effect upon the excised uteri of animals, inducing powerful contractions, especially in the pregnant uterus. The active constituent is related to but not identical with **β-Iminazolyl Ethylamine**. We may assume with probability that the hypertrophy of the gland which accompanies pregnancy may result in the presence of this substance in increasing amounts in the blood-stream until, finally, the hyperirritability of the uterus, with the assistance of the second active substance about to be noted, reaches a stage culminating in contractions which expel the fetus.

The second factor which operates in the direction of promoting contraction of the uterus, is the presence of a substance in the **Colostrum** or first secretion of milk, which causes contractions of the pregnant uterus. In fact abortion has been brought about in pregnant cattle before the normal period of delivery, by injections of colostrum from a normal cow. Colostrum differs in many respects from the milk which is subsequently secreted. This will be clear from the following analyses of cows' milk, by König.

Per 1000.	Water.	Solids.	Casein.	Other protein.	Fats.	Sugar.	Salts.
Colostrum . .	746.7	253.3	40.4	136.0	35.9	26.7	15.6
Milk	871.7	128.3	30.2	5.3	36.9	48.8	7.1

It has been recognized from a remote period that colostrum has a cathartic action upon the infant, so that the substance inducing uterine contractions may possibly be a general muscular stimulant. Its chemical nature is, however, unknown.

THE CHEMICAL REGULATION OF METABOLISM.

The activities of our various tissues are so closely interwoven with one another, and the various organs of the body are so intimately dependent upon one another for the raw materials which they elaborate into finished products, or the disposal of waste products which might otherwise be deleterious to the well-being of the whole bodily economy,

that the complete analysis of the coördinate factors of our total metabolism would involve a survey, necessarily incomplete at the present stage of our knowledge, of the whole gamut of physiological activities. Without attempting to embark upon such an ambitious review, there are certain outstanding factors in the regulation of metabolic activity which compel our attention here, because the regulatory action which they exert would appear to constitute the prime function of the tissues concerned.

FIG. 23.—Cachexia strumipriva following total extirpation of thyroid; eleven years after operation. (After Kocher.)

The most striking effects upon the general metabolism of the body are those which are exerted by the tissues of the **Thyroid.** Our attention was first drawn to the importance of this gland in the bodily economy by pathological conditions which are endemic in certain localities and sporadic in all human communities. The disorders resulting from improper functioning of the thyroid fall into two main classes, those namely, which result from subnormal development or activity of the gland, and those which result from its overactivity.

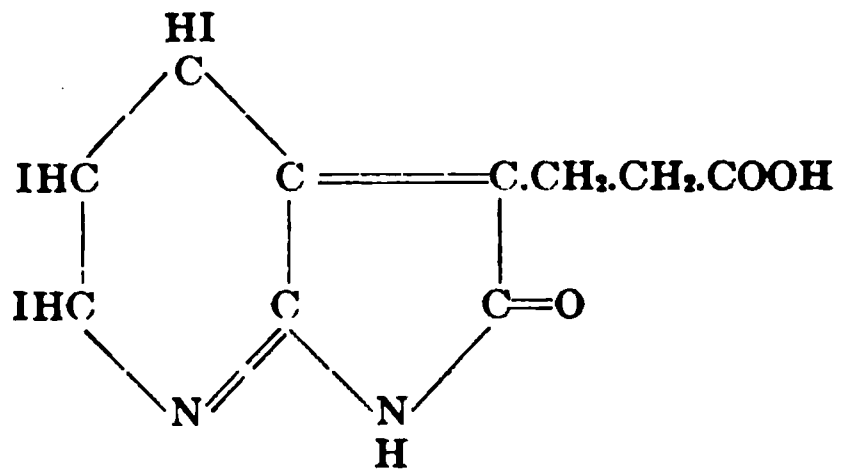
The condition of **Myxedema** arises when the thyroid fails to develop

properly, or, in later life, is extirpated, or injured by degenerative changes. If the failure of the gland occurs in childhood, intellectual development is arrested, and the condition known as **Cretinism** supervenes. The expression is idiotic, the skin is greatly thickened through the overdevelopment of connective tissue, and the features are consequently coarsened and brutalized. In adults, extirpation or destruction of the gland by disease results in similar symptoms (Figs. 23 and 24) but the intelligence, although it becomes very sluggish, remains

FIG. 24.—The same patient as in Fig. 23, five months after thyroid administration. (After Kocher.)

far above the level of an idiot. The temperature of the body is subnormal, the total metabolism is much reduced, and the daily nitrogen output is subnormal. These conditions, if taken in hand early, are completely curable by the administration of extracts or dried preparations of the thyroid gland. This is, in fact, the most completely successful instance of organotherapy to which we are as yet able to point, and provided the administration of the glandular preparations in appropriate dosage be continued, individuals who would otherwise exhibit most extreme symptoms of the disorder remain in satisfactory health, with unimpaired intelligence and vigor.

The active and remedial constituent of the gland is associated with the **Iodine** which the thyroid contains and which distinguishes it chemically from all other tissues of the body.[1] While the iodine-content of the thyroid varies very much, not only in different species of animals, but in different individuals of the same species, yet the minimal content of iodine which is consistent with normal functioning of the gland is very nearly constant and, on the other hand, the remedial value of a thyroid preparation tends to be proportionate to its iodine-content. The nature of the active iodine compound has been the subject of very many and extensive investigations. The experiments of Oswald showed that the active substance, as it exists in the glandular tissue, is either an iodized protein or closely associated with a protein which he termed **Thyreoglobulin.** Bauman found, however, that the partial hydrolysis of Oswald's thyreoglobulin by means of sulphuric acid did not destroy its therapeutic activity, but that a fraction of the hydrolytic cleavage-products which he termed **Iodothyrin** retains the original activity of the thyreoglobulin. This substance, according to von Fürth, is related to the "humin" substances which form in acid hydrolyses of protein in the presence of carbohydrate radicals, and are considered by Gortner and Blish to arise from the **Tryptophane** groups of the protein molecule. This fact has received peculiar significance as a result of the recent researches of E. C. Kendall who has succeeded in still further fractionating the hydrolytic cleavage-products of thyreoglobulin without destroying its therapeutic activity. By hydrolyzing thyreoglobulin in alkaline alcohol two groups of products are obtained. The one group is insoluble in dilute acids, the other is soluble. The acid-soluble substances are physiologically and therapeutically inert and they contain very little iodine. The acid-insoluble substances contain a high proportion of iodine, and are physiologically and therapeutically potent. By further fractionation Kendall obtained a white crystalline product containing 60 per cent. of iodine, which was very active therapeutically and proved to be a derivative of **Indol,** being therefore related to tryptophane. Kendall believes that this compound which he designates **Thyroxin** is a tri-iodo-oxy-indol-propionic acid, and has tentatively suggested the following constitutional formula:



[1] The alleged presence of iodine in the pituitary gland has not proved possible to confirm.

The administration of an excess of thyroid tissue to animals or man is accompanied by a very marked acceleration of metabolism. On a normal mixed diet the total heat-output may be raised 100 per cent. The effect of this enhanced metabolism is to cause a reduction of weight due to loss of tissue, and especially of fat, and it is for this reason that thyroid extract is the chief and only effective constituent of a variety of **Obesity-cures**. Unfortunately, however, the nitrogenous output is proportionately increased, so that the obese person loses not only fat, but also tissue-protein, which he frequently can ill afford to spare. Furthermore, distressing or even dangerous cardiac symptoms are liable to supervene with overdoses of thyroid extract, or even with moderate doses if the thyroid of the patient is normally active, so that the unrestricted use of thyroid preparations by the public is attended by serious danger.

The stimulation of the destruction of nitrogenous tissue-constituents which follows the administration of thyroid is extremely striking. Thus, Rhode and Stockholm have found that in dogs receiving only sugar as a diet, so that the nitrogenous output was minimal, the output was increased fifty per cent. by so small an amount as 0.10 to 0.15 grams of dried thyroid tissue per kilogram body-weight of the animals. Arguing chiefly from the fact that his crystalline active fraction reacts with amino-acids, combining with the amino-group and liberating carbonic acid, Kendall has advanced the view that the thyroid secretion catalyzes the process of **Deaminization** of amino-acids. The power of deaminizing amino-acids is known to be shared by all the tissues and the stimulating effect of thyroid extract is likewise common to all tissues. The question is an extremely difficult one to decide, for when we recollect that the proteins of the tissues stand in a relation of equilibrium to the reserve amino-acids which they contain, and that these in turn are in equilibrium with the amino-acids circulating in the blood it is evident that anything tending to break down the amino-acids which have not yet become integral living tissue must also indirectly lead to the breaking down of tissue-protein, and the stimulation of endogenous catabolism. In support of Kendall's theory, however, may be cited the facts that hyperthyroidism, as in exophthalmic goiter, is aggravated by a high protein diet, and that the effects of thyroidectomy are more serious in carnivorous than in herbivorous animals.

A remarkable effect of administration of thyroid tissue to mice is the extraordinarily increased tolerance for **Acetonitrile** to which it leads. Reid Hunt has found that if 0.1 milligrams of dried thyroid tissue be administered to mice on ten successive days, they will withstand ten times the normal lethal dose of acetonitrile, administered subcutaneously, and indeed he proposes this enhanced tolerance to a specific substance as a test for the activity of various thyroid preparations. The significance of this effect is, however, uncertain because it is not universal; in fact in such a closely allied animal as the rat,

administration of thyroid tissue, so far from enhancing the tolerance for acetonitrile, actually renders the animals more sensitive than usual to intoxication by this poison.

Hyperthyroidism occurs spontaneously in the condition known as **Basedow's Disease**, or **Exophthalmic Goiter.** This condition is accompanied by enlargement of the gland and a marked increase of secreting cellular elements, the interspaces filled with colloidal material which are characteristic of the structure of this gland being much reduced in size. There is a greatly enhanced metabolism, the calorific output being frequently twice the normal; there is a slow progressive loss of weight, incoördination of the heart-beat (**Tachycardia**), the temperature is supernormal, the nervous system hyperirritable, and the blood-pressure is usually abnormally high. The rate and intensity of living is in fact increased in all its aspects, and frequently to a dangerous extent. The administration of thyroid preparations, or in fact of any iodine-containing substance, leads to a reduction of the **Hyperplasia** of the epithelium of the gland, and an increase in the quantity of colloidal material, that is, to a return toward the normal structure. It is a question whether the symptoms of Basedow's disease are altogether attributable to hyperfunctioning of the gland. The remedial effects of iodine would point rather toward a deficiency of the iodine-containing principle as the origin of the hyperplasia of the secreting epithelium which characterizes the disease. In fact the iodine content of the hyperplastic gland may actually be below normal, and a similar condition may be aroused in the residue by excision of a considerable portion of the gland, as if the effort of a small part of the thyroid tissue to assume the functions of the whole stimulated a proliferation of the epithelial elements. On the other hand it must be recollected that a deficient content of any substance in a secreting gland does not necessarily mean that the production of the substance is diminished; it may merely mean that its rate of discharge from the gland is abnormally high, so that it has no opportunity to accumulate within the tissues of the gland itself.

The prevalence of **Myxedema** and goiter in certain geographical areas and particularly in mountainous or hilly regions, and the comparative rarity of such conditions elsewhere, has led us to ascribe the endemic forms of thyroid disease, directly or indirectly, to localized physiographical or geological conditions. Even in the days of Marco Polo, the prevalence of **Goiter** was attributed to a peculiar quality of the water in the localities affected,[1] and this impression still prevails, both in medical and in lay circles. Notwithstanding the clue this offered, however, it has not yet proved possible to establish the nature

[1] "Departing from thence" (Samarcand) "you enter the province of Karkan . . . The people . . . are in general afflicted with swellings in the legs and tumors in the throat, occasioned by the quality of the water they drink." The "swellings in the legs" are attributable to a nematode worm, *Filaria medinensis* of which the "*carrier*," or intermediate host, is a minute fresh-water crustacean, *Cyclops*.

of the abnormality in drinking-water which causes disorders of the thyroid. It cannot even be definitely stated whether the abnormality consists in the presence of an infecting agent, or in a chemical component or its absence. The numerous circumscribed and yet widely separated areas of endemic occurrence, however, speak against the view that the disease is communicated by an infecting organism. The goiter which occurs among fishes in hatcheries, has been traced to overfeeding with a high protein diet.

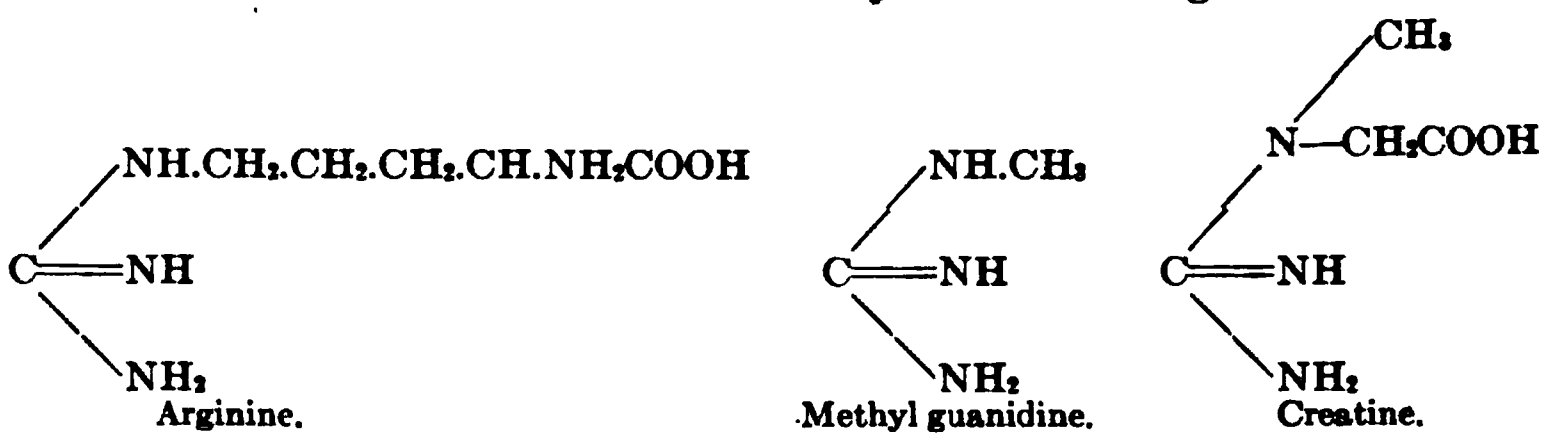
Lying just above the thyroid, or, in some animals, imbedded in the thyroid tissue, are a variable number (two pairs in man) of small glands, known collectively as the **Parathyroids.** Structurally they differ essentially from the thyroid and evidently they also differ from the thyroid very decisively in function, for their excision leads to quite a different sequence of events from those which follow thyroidectomy. The removal of the parathyroids, if complete, results in acute neuromuscular symptoms which are collectively designated **Tetany,** and which resemble very closely a condition which not infrequently arises spontaneously in young children. For a little time succeeding parathyroidectomy, no abnormalities appear, but within forty-eight hours tremors are observed in the extremities, followed by involuntary contractions of more and more muscles of the body until, finally, convulsions supervene, terminating after several days in death. The condition is completely relieved, according to W. G. Macallum, by the administration of **Calcium Salts,** and for this reason it was thought probable, for some time, that the special function of the parathyroids consists in the regulation of the **Calcium Metabolism.** Many facts, however, speak against this view. In the first place observers are not agreed that the excision of the parathyroids leads to increased excretion of calcium or a reduction of calcium in the blood and tissues, and in the second place other disturbances of metabolism to which attention has been directed in recent years offer a more probable origin of the neuromuscular symptoms. The remedial effect of calcium salts is regarded merely as an example of the general action of calcium in reducing the irritability of nerve fibers. On the other hand some disturbance of the calcium metabolism unquestionably accompanies parathyroidectomy, for it has been found by Erdheim that parathyroidectomy in rats (probably not complete) leads to deficient dentine-formation in the teeth of the operated animals, and Erdheim and Carrel have found that callus-formation in injured bones is delayed by parathyroidectomy.

The effect of parathyroidectomy upon the nitrogenous metabolism is very marked. The output of **Ammonia** is much increased, and for this reason Kendall and others have suggested that the parathyroids control the transformation of ammonium carbonate into **Urea,** which is the normal end-result of the deaminization of amino-acids, and occurs primarily in the liver. There is a decided **Alkalosis** or increased alkalinity of the blood in parathyroidectomy, and the symptoms may be alleviated by the injection of acids. On the other hand it has not

proved possible to induce tetany by injections of ammonia or ammonium carbonate.

The urine of children between the ages of two and fifteen normally contains **Creatine**, which is absent from the urine of adults,[1] and it is between these ages that children are most liable to develop symptoms of tetany. On the other hand the content of creatine in muscular tissues is definitely connected with their **Tonus** or degree of tonic contraction and is increased by all measures which increase tonus. It has therefore been suggested by many observers that the tetany arising from parathyroidectomy may originate in a disturbance of the normal metabolism of creatine. In this connection it is of especial interest to note that Landois and Maxwell have found that while the gray matter of the motor-areas of the cerebral cortex is remarkably insensitive to the ordinary chemical stimuli which increase the irritability of nerve-fibers (calcium precipitants), it is powerfully stimulated by applications of creatine, with the effect of inducing convulsions. It has not, however, proved possible to induce tetany in animals by injections of creatine.

Creatine is methyl guanidine acetic acid, and is therefore related to the amino-acid, **Arginine**, the relationship of arginine, methyl guanidine and creatine to one another is shown by the following formulæ:



Methylguanidine and **Dimethyl Guanidine** occur in small amounts in blood, muscular tissues and urine. It has recently been shown by N. Paton that the quantity of methylguanidine in the blood and urine is decidedly increased after parathyroidectomy in animals, and in the spontaneous tetany which occurs in children. The following figures are illustrative:

Guanidine + Methylguanidine in milligrams per liter.

A. BLOOD.

DOGS.

Normal.	Parathyroidectomy.
1.00 (average of 5)	8.7 (average of 8)

B. URINE.

DOGS.

Normal.	Parathyroidectomy.
0.25 (average of 6)	1.1 (average of 6)

CHILDREN.

Normal.	Idiopathic tetany. (Average of 3 cases)	
0.12 (average of 8)	Active tetany	0.58
	Latent tetany	0.38
	Recovery	0.12

[1] Occasionally present in the urine of women.

The subcutaneous or intravenous injection of **Guanidine** or **Methylguanidine** was found by Paton to lead to marked symptoms of tetany. Previous observers had established the fact that guanidine causes fibrillary twitchings of muscular tissue through stimulation, followed by paralysis of the myoneural junctions, and Fühner, in 1906, demonstrated that this action is antagonized by calcium salts. The origin of parathyroid tetany would therefore appear to reside in a disturbance of nitrogenous metabolism, and especially in the metabolism of the guanidine derivatives. The aggravation of symptoms which accompanies the administration of a high meat-diet is thus accounted for. Whether the parathyroids control the metabolism of other nitrogenous constituents of the diet besides those which contain a guanidine nucleus, is unknown, but the alkalosis which accompanies parathyroidectomy suggests that the products of metabolism which the parathyroids remove or elaborate are strongly basic substances such as might be derivable from the decomposition of **Diamino Acids**, of which, of course, arginine is an example.

It has recently been shown by Uhlenhuth that tetany may be induced in amphibian larvæ which do not possess parathyroids (Amblystoma); by the administration of thymus tissue, and he suggests that the function of the parathyroids is to remove or render non-toxic substances produced by the **Thymus**. This would also explain the prevalence of tetany in children, since the thymus degenerates as maturity is attained. While this is very possible, it must also be remembered that the thymus is unusually rich, among animal tissues, in **Thymus Nucleic Acid**, which yields **Guanine** among its decomposition-products. Now guanine, when oxidized, yields, among other products, guanidine, so that the tetany observed by Uhlenhuth may have had a dietary rather than a specific glandular origin.

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PART IV.

THE CHEMICAL PROCESSES WHICH UNDERLIE AND ACCOMPANY LIFE-PHENOMENA.

CHAPTER XVII.

PROCESSES INFERRED FROM DIRECT OBSERVATION.

THE INTERMEDIATE METABOLISM OF THE CARBOHYDRATES: MUSCULAR CONTRACTION.

We have seen, in considering the chemical regulation of the respiratory movements, that the energy-expenditure in muscular exertion is derived from oxidations. This follows immediately from the low heat-value of the hydrolyses which occur in the body, and which render them insufficient sources of energy, and from the greatly increased consumption of oxygen and output of carbon dioxide which accompanies the performance of muscular work.

It remains to consider, however, what class of foodstuffs undergoes the oxidations which release muscular energy. That **Carbohydrates** afford a proportion of the necessary heat-units is evident from the fact that during the performance of muscular work the **Glycogen** which is normally stored up in muscular tissues, is greatly diminished in quantity, and even the further reserve which is stored up in the liver becomes much reduced by the performance of severe and long-sustained muscular work. The potential energy contained in these reserves of glycogen is very considerable. Thus the liver of a man, when fully stocked with glycogen, contains about 150 grams of this polysaccharide, while the muscles, at rest and after feeding, contain a like amount. The total available reserve of carbohydrate material in the body is therefore about 300 grams, having a heat-value of 4.1 calories per gram or 1230 in all. If only one-fifth of this potential energy were converted into mechanical work, its remainder being dissipated as heat, it would lift a weight of one hundred tons to a height of over three feet.[1]

The exhaustion of glycogen by the performance of **Muscular Work**

[1] The equivalent of one calorie in mechanical work is 426.5 kilogram-meters.

may be observed in a variety of ways. In the first place we may excise the two corresponding leg-muscles of a frog, analyze one to serve as a standard for resting muscle, and stimulate the other with a tetanizing current until exhaustion supervenes, and the muscle will contract no longer, and then repeat the analysis upon this exhausted muscle. The content of glycogen in the stimulated muscle is invariably found to be lower than in the resting muscle, as much as fifty per cent. of the glycogen being generally found to have disappeared.

Another way of approaching the problem is to cut the motor-nerves supplying one set of leg-muscles and, after the lapse of a definite period, to compare the glycogen-content of these muscles deprived of nervous connections with the glycogen-content of the corresponding normal muscles on the other side of the body. The muscles of our skeleton, while their nervous connections remain intact, are in receipt of constant slight nervous stimuli, insufficient to elicit actual contractions, but maintaining a condition of **Tonus** or constant tension which is a favorable precedent to rapid and forcible movements. This tonic contraction of the muscles of the skeleton consumes energy, not in the performance of external work, it is true, but in the performance of **Internal Work**; the overcoming of resistances analogous to friction or to the resistance to extension which is displayed by a liquid surface. This tonus and its resultant expenditure of energy are prevented by cutting off the stimuli which maintain it, so that a muscle with its motor-nerves severed, relaxes, and consumes less energy than a normal muscle with its nervous connections intact. Corresponding with this we find that the glycogen reserves in the paralyzed muscles tend to accumulate and to exceed the glycogen-content of the innervated muscles on the opposite, unoperated side of the body. This is clearly shown by the following determinations by Marcuse upon rabbits, the sciatic and crural nerves having been severed upon one side:

Experiment Number	Percentage of glycogen. Paralysed muscles.	Innervated muscle.
1	0.748	0.539
2	0.749	0.461
3	0.589	0.395
4	0.542	0.341
Average	0.657	0.434

The glycogen-reserve, through lack of expenditure, was therefore increased fifty per cent. in the paralyzed and demobilized muscles.

Again, we may compare the glycogen-content of all the tissues in two similar animals, in the one after a period of rest, and in the other after a period of intense muscular exertion, and we obtain again the same result, namely a disappearance of glycogen with the performance of muscular work. Thus Külz forced a large and well-fed dog, weighing 45.5 kilos, to draw a heavy cart for nine hours and forty minutes. The animal was then killed, and the total glycogen-content of all its tissues

was determined. Fifty-two grams of glycogen were obtained, corresponding to 1.16 grams of glycogen per kilogram of body-weight. A normal well-fed dog of similar dimensions contained 3.8 grams of glycogen per kilogram of body-weight. Even after four weeks of starvation a similar dog was found to contain 1.5 grams of glycogen per kilogram of its body-weight, so that somewhat less than ten hours of severe muscular exertion reduced the glycogen reserves of the body to a greater extent than four weeks of sheer starvation.

So far, then, we have proved that muscular energy may be and is derived, in part at least, from the consumption of carbohydrate materials. The question now remains, what *proportion* of the energy of muscular work is provided by the carbohydrates of the food? For while the experiments which we have cited show that a part, and probably a large part of the energy expended in muscular work is certainly derived from carbohydrates, they do not preclude the possibility that an important proportion of the necessary heat-units may be supplied by other foodstuffs, for example by **Proteins.**

This question was answered as early as 1865 by a classical experiment which was performed by Fick and Wislicenus. These observers ascended Mount Faulhorn, climbing to a height of 1956 meters above the starting-point. For seventeen hours before they started, during the six hours occupied in the ascent, and for six hours following the completion of the ascent they consumed no food which contained nitrogen. The urine passed during the ascent and in the six hours succeeding the ascent was collected and from its nitrogen-content the total quantity of body-protein which had been decomposed was estimated. It was found that Fick had decomposed 38.3 grams of protein while Wislicenus had decomposed 37.0 grams. Now if we assume, which, of course is not the fact, that all of the protein was decomposed so completely as to produce the end-products of perfect combustion, namely CO_2, H_2O and nitrogen, this quantity of protein would have liberated 250 calories, equivalent, if it were wholly converted into mechanical work, to 106,000 kilogram-meters. But Wislicenus, for example, weighed 76 kilograms, and the work which he actually performed in the mere effort of raising his body through 1956 metres was $76 \times 1956 = 148{,}656$ kilogram-meters, so that upon the most excessively liberal computation the protein which was decomposed during and after the ascent could not possibly have furnished the energy consumed in the ascent. As a matter of fact, the actual yield of calories when protein is burnt in the body is much less than that which would be derived if combustion were complete, for instead of nitrogen being formed the oxidation stops with the production of **Urea** which has a very considerable heat-value of its own and which is voided from the body and not utilized. Furthermore no machine is known, not even a living machine, which can quantitatively convert heat into mechanical work. In fact actual measurements have shown that only twenty per cent. of the heat-value of foods is, as a rule,

available for the production of mechanical work. If we apply these various corrections to the above estimate of the work available from the proteins destroyed by Fick and Wislicenus, we find that it actually amounts to only 13,000 kilogram-meters, or less than nine per cent. of the work required merely to lift the weight of their bodies to the top of the mountain. Now it must be remembered that the ascent of their bodies was by no means the whole of the mechanical work which was performed by these experimenters, for, apart from the tonus of their skeletal muscles, the work of the secretory and excretory organs, and the movements of the digestive canal, expenditures of energy that cannot very easily be computed, their circulations had to be maintained by the beating of their hearts and their respiratory movements by contractions of the diaphragm and intercostal muscles. These sources of expenditure of energy alone can be estimated to have accounted for no less than 30,000 kilogram-meters of work during the ascent of the mountain. All the energy actually procurable from the protein they decomposed, therefore, would not have half sufficed to maintain the respiratory movements and the heart-beat, leaving nothing over whatever for the ascent of the mountain. The proportion of muscular energy furnished by the proteins must therefore have been very small.

That under normal conditions the whole of the energy consumed in muscular exertion is derived from non-protein sources, is rendered very probable by the discovery of Voit, that work upon the treadmill by a dog fed upon mixed rations does not increase the nitrogen output. Not only is the total nitrogen output unaffected by muscular work upon a mixed diet, but the entire **Protein Metabolism** pursues its normal course, undisturbed by the large expenditure of energy which is occurring. This is shown by an experiment by Shaffer, who investigated the urine of a man fed upon a purine-free diet, containing a minimal allowance of nitrogen, in three different periods, namely, a rest-period of six days which he spent in bed; a normal period of five days which he spent in performing light work about the laboratory, and a work period of four days in which he added to the laboratory work long daily walks. The following were the results obtained:

Period.	Food.		Urine.					
	N. grams.	Calories.	Total N.	Nitrogen present as:				Sulphur.
				Ammonia.	Creatinin.	Uric acid.	Undetermined.	
I. Rest .	5.9	2300	4.77	0.35	0.605	0.11	0.35	0.438
II. Normal	6.0	3000	4.40	0.38	0.600	0.106	0.42	0.424
III. Work .	5.9	3200	3.94	0.42	0.560	0.12	0.42	0.414

The question arises, however, whether, if placed under practical compulsion to do so, by the scarcity or absence of other source of

energy, the muscular tissues may not be able to utilize proteins for the performance of mechanical work. Experiments of Kellner, conducted upon horses, render this very probable, for this observer found that while muscular work upon a mixed diet, as Voit had previously shown, does not increase the nitrogenous output of the horse, yet muscular work upon a diet which contained an insufficient allowance of carbohydrates did result in a notable increase of nitrogen elimination. This fact may be paralleled by the oft-repeated observation that while **Bacteria** will preferably obtain their energy from carbohydrates in the culture-medium, yet if these be insufficient in amount, proteins are attacked and energy is derived from the hydrocarbon radicals which they contain, nitrogenous fragments being split off as by-products of the process.

Now proteins, being an abnormal source of muscular energy, may very possibly give rise to some unusual products when necessity compels their utilization for this exceptional purpose. We recognize that the protein metabolism of muscular tissues is peculiar. The abundance of **Creatine** in the muscles and the presence of **Methylguanidine, Dimethylguanidine, Carnitine** and other physiologically active nitrogenous bases in muscular tissues show that the degradation of protein in these tissues does not follow the channels normal to other tissues, and arouses the suspicion that rapid and extensive breaking-down of muscle-proteins might lead to the production of toxic bases in dangerous amounts and to notable physiological disturbances. We are reminded, in this connection, of the fact that the dangerous toxemia of pregnancy, **Eclampsia,** is often accompanied by sudden involution (degeneration) of the muscular tissues of the uterus. Nor are there wanting facts which tend directly to show that extreme muscular exhaustion upon a high protein diet may be dangerous. The experiences of Mawson and Mertz in the Australian Antarctic expedition of 1912–1913, which culminated in the tragic death of Dr. Xavier Mertz, may be instanced. In severe antarctic weather and heavily crevassed country, involving extraordinary expenditures of energy to maintain bodily heat and make progress over the ground, at a distance of three hundred miles from headquarters, these explorers, through loss of a companion and a sledge in a crevasse, found themselves with a bare one and a half weeks' food for themselves, and none at all for the dogs. They started to walk back to their headquarters, killing the dogs from time to time and consuming their necessarily excessively lean flesh. After eighteen days Mertz began to fail, and during several days expressed especial aversion to the dogs' meat; he displayed great muscular weakness, and complained of violent abdominal pains from which Mawson also suffered. Seven days later symptoms of central nervous intoxication appeared. The following are notes from Mawson's diary:

"January 7.—It was a sad blow to me to find that Mertz was in a weak state and required helping in and out of his bag. He needed rest for a few hours at least before he could think of travelling. I have

to turn in again to kill time and also to keep warm for I feel the cold very much now."

"At 10 A.M. I get up to dress Xavier and prepare food, but find him in a kind of fit. Coming round a few minutes later, he exchanged a few words and did not seem to realize that anything had happened . . ."

"During the afternoon he had several more fits, then became delirious and talked incoherently until midnight, when he appeared to fall off into a peaceful slumber. . . After a couple of hours, having felt no movement from my companion, I stretched out an arm and found that he was stiff."[1]

These are not symptoms of mere inanition. Definite intoxication was also present, and it appears not improbable that the extraordinary exertions necessitated by their situation, carried out as they were upon an almost exclusively protein diet, may have led to the abnormal disintegration of food- and tissue-proteins by the muscular tissues, with the production of poisonous nitrogenous, fragments.

The employment of a high protein diet as a preparation for muscular exertion and endurance is therefore in the highest degree irrational, more especially since the rate of loss of heat from the body on a protein diet is diminished, so that the cooling necessary for the maintenance of prolonged bodily effort is rendered more difficult than usual. The only possible ground for the formerly popular dietary of beefsteak for athletes is the fact that on a diet purely of flesh the muscular machine is more efficient, *i. e.*, produces less heat per unit of external work performed. In fact in a dog fed upon pure flesh Pflueger obtained the highest work-yield that has ever been observed, nearly fifty per cent. of the heat-value of the food appearing as mechanical work. For a short, sharp "dash" or brief effort, therefore, a high protein diet may possess advantages, but for prolonged extreme exertion a mixed diet containing an exceptionally abundant allowance of carbohydrates is the only rational prescription. This is, in fact, the actual dietary which, in the absence of suggestion or direction, is voluntarily chosen by those classes and groups of individuals whose mode of earning a living compels great and sustained muscular effort.

The normal source of muscular energy is therefore the carbohydrates of the dietary. That the **Fats** may also be utilized for this purpose is evidenced by the fact, first established by Rubner, that fat and carbohydrate are **Isodynamic Foodstuffs**, *i. e.*, that equicalorific amounts of these substances can replace one another in the diet. There has been some discussion of the question whether or not the fats are *directly* utilized for the performance of work, or whether they may not have to undergo a preliminary transformation into carbohydrates. This question has been experimentally investigated by Zuntz, who found that when carbohydrates predominate in the diet the total amount of

[1] The Home of the Blizzard, Sir Douglas Mawson, London, 1915, vol. i, pp. 258–259.

energy liberated by the body (work plus heat) corresponds to 9.33 small calories[1] for every kilogram-meter of work performed, while if the carbohydrate be replaced by fat, the total liberation of energy is 10.37 calories for the same amount and kind of work. Now 2.35 small calories are equivalent to one kilogram-meter of mechanical work, so that on a carbohydrate diet 25 per cent. of the excess of energy-dissipation due to work was actually converted into mechanical work, and on a fat diet 22.7 per cent. There is thus little difference of efficiency whether fats or carbohydrates furnish the source of energy. Now if fats had first of all to be converted into carbohydrates, before they could be utilized for work, a great deal of oxygen would have to be introduced into the molecule, since the fats contain a much higher proportion of hydrogen to oxygen than the carbohydrates. If all this preliminary oxidation were unavailable for the production of muscular energy, not less than 29 per cent. of the energy of the fat would be wasted, and we would expect the performance of mechanical work on a fat diet to be only two-thirds as efficient as upon a carbohydrate diet. It is highly probable; therefore, that fats undergo but little preliminary modification before they are available for muscular work. They are not the first choice of the muscles, however, if all dietary materials are available, carbohydrates are used first. Fats are pressed into the service when carbohydrates begin to fail, and proteins form a last resource.

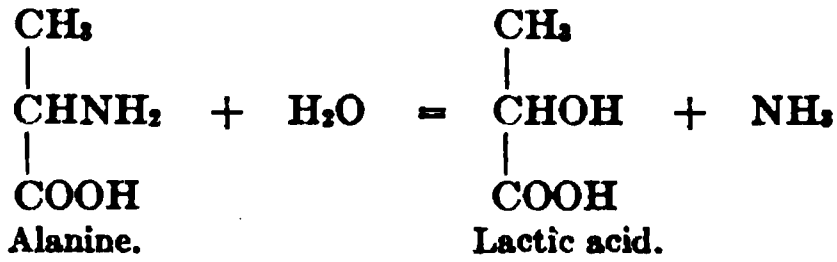
The performance of muscular work involves a considerable increase of oxygen-intake, and carbon-dioxide output. The final products of muscular exertion are therefore carbon dioxide and water. The oxidation of glycogen or its hydrolytic cleavage-product, glucose, is not accomplished in a single step, however. Intermediate products are transiently formed, and of the nature of many of these we can only form conjectures which, however, are gradually becoming more and more clearly defined as persistent research reveals, one after another, the various substances which may arise from the oxidations of glucose in the animal body. One of the first of these intermediate products to be clearly recognized was, however, **Lactic Acid.**

The lactic acid which is found in muscular tissue is not the ordinary racemic acid which may be obtained by synthesis in laboratory-glassware. It is the dextrorotatory acid, or **Sarcolactic Acid**:

which, when pure, forms a viscous, acid syrup, forming crystalline salts with a variety of bases. The zinc salt is the one usually employed for the isolation and estimation of lactic acid in muscular tissues.

[1] The small calorie is the heat required to raise the temperature of 1 gram of water one degree. The large calorie is the heat required to raise the temperature of a kilogram of water one degree.

There has been some discussion of the question whether the lactic acid of muscular tissues actually arises from the partial oxidation of carbohydrates or whether it may not, on the contrary arise from **Proteins**, as, for example, by the deaminization of the **Alanine** radical of proteins:



Alanine. **Lactic acid.**

While such an origin of sarcolactic acid must be admitted to be possible, yet it is more probable that the major part of the lactic acid produced by the muscular and other tissues of the body, arises from a carbohydrate source. Thus Mandel and Lusk have shown that in **Phosphorus-poisoning** there is a great increase in the lactic-acid output in the urine. If, however, the body has previously been drained of its carbohydrate reserve by inducing **Glycosuria** through the administration of **Phloridzin**, then phosphorus-poisoning results in no hyperproduction of lactic acid.

The lactic acid in excised muscles of the frog rapidly diminishes on standing. This is due to its oxidation by the muscle-tissues. Now the oxidation of lactic acid is evidently a more difficult step to accomplish than its production from glucose or glycogen, for, if the oxygen supplied to the muscles be interfered with by asphyxia, by inhalation of air poor in oxygen, or by poisoning with **Carbon Monoxide**, the lactic-acid content of the tissues and of the blood and urine is enormously increased.

One of the characteristics of extreme muscular **Fatigue** is the stiffening and inextensibility of the muscles which ensues. After death the **Rigor Mortis** or postmortem stiffening of the muscles occurs with extreme rapidity if the animal has immediately prior to death been engaged in extreme and prolonged muscular exertion. The stiffening and increased opacity of the muscles which occurs after extreme fatigue or death is due to the coagulation of certain proteins which the muscle-fluids contain, the semifluid **Muscle-plasma** being converted into a jelly.

It was found by Halliburton that if muscles be frozen and minced and then subjected to pressure at a temperature slightly above freezing, an opalescent fluid is obtained which clots spontaneously upon warming to a little above bodily temperatures, or upon standing for some time at room-temperatures. According to von Fürth the gelatinization of this fluid is due to changes which occur in two proteins, the one a globulin, **Myosin** and the other an albumin, **Myogen**. The myogen fraction is much the more abundant of the two. Upon heating or acidification these soluble proteins are transformed, respectively, into **Myosin Fibrin** and **Myogen Fibrin**. The process is not reversible; the jelly cannot be liquified by cooling or by neutralization. It is believed that the partial gelatinization of these proteins which constitutes rigor in

muscles is brought about by the lactic acid and even, in part, by the carbon dioxide which accumulates in fatigued muscles.

The **Creatine** content of muscular tissues is not decisively affected by muscular work. It appears that the increase, if any, is very slight, a fact which corresponds to the subordinate part which is normally played by proteins in the development of muscular energy. Nevertheless Van Hoogenhuyze and Verploegh have found a definite increase of creatine in muscular tissues after severe work, provided the work was performed by starving animals. In other words if protein is of necessity employed by the muscles as a source of energy, then creatine is numbered among the chemical products of muscular work. The production of creatine appears, however, to bear an especial significance in relation to muscular **Tonus**; any agent tending to increase the tonic contraction of the muscles leading to an increased creatine-content. Thus the creatine-content of the muscles is increased by drugs such as **Cinchonine** which increase tonus, and in pregnancy the creatine-content of the muscular tissues of the **Uterus** is very greatly increased.

THE INTERMEDIATE METABOLISM OF THE FATS; DIABETES.

The normal products of the oxidation of the fats and sugars are finally, as we have seen, carbon dioxide and water. In animals with normal metabolism, but few of the intermediate products of oxidation can be perceived, because the various stages are passed through rapidly when the oxidation is once begun, and intermediate products of the process, therefore, have no opportunity to accumulate. One stage which is easily recognizable is that afforded by the production of **Lactic Acid** because the next step in the oxidative processes is evidently accomplished with relative difficulty, so that a proportion of this product accumulates in the tissues, especially if the oxidative processes are interfered with so as to increase the difficulty of further transformation. Our knowledge of other stages, in the oxidation-processes of the body is, however, very largely derived from an experiment which is performed for us by nature in the disease or group of diseases known as **Diabetes Mellitus.**

Glycosuria, the excretion of sugar in the urine, may be induced by the injection of physiologically unbalanced **Salt Solutions** and particularly by solutions containing **Magnesium Salts.** The origin of this glycosuria, whether it arises from an unusual discharge of sugar from the muscles or the liver, or from an increased permeability of the kidneys for sugar, has not as yet been ascertained. A glycosuria without any accompanying **Glucohemia,** that is, without any increase in the normal percentage of sugar in the blood, may be induced by the administration of the glucoside **Phloridzin.** This glycosuria is evidently due to an alteration of the normal **Permeability** of the kidney for sugar. The epithelium of the normal kidney interposes an impassable barrier

to the passage of sugar into the urine provided that the sugar in the blood does not much exceed the normal concentration of 0.10 to 0.15 per cent. After treatment with phloridzin, however, this barrier breaks down. The normal sugar-content is drained out of the blood, and the liver and muscles, in the endeavor to restore the normal equilibrium between **Glycogen** and **Glucose**, release glucose continuously to the blood, so that the ultimate result is the drainage of the carbohydrate reserves of the body. That the effect is a purely local one upon the epithelium of the kidneys is shown by the fact that if the phloridzin be supplied only to one kidney by perfusion into the corresponding renal artery, that kidney, but not the other, will eliminate glucose. It has been supposed that phloridzin, being a glucoside, acts as a carrier of glucose across the kidney-epithelium, liberating glucose on the one side and combining with it upon the other, but of this we have no definite proof.

Yet again, glycosuria may result, temporarily, from an excessive ingestion of carbohydrates, and particularly of sugars. This form of glycosuria, known as **Alimentary Glycosuria** is not serious unless, indeed, it occurs too readily, when it may indicate a slight or incipient diabetes. It is stated by Cushing that alimentary glycosuria tends especially to occur in conditions of **Hyperpituitarism** or overactivity of the pituitary gland, of which condition, in fact, he considers a readily elicited alimentary glycosuria to afford confirmatory diagnosis. In the opposite condition of **Hypopituitarism** he finds, on the contrary, an extraordinary tolerance for ingested sugars and alimentary glycosuria fails to appear after a dosage of glucose or levulose which, in normal individuals, would inevitably be followed by an excretion of sugar in the urine. Other observers, while confirming Cushing's observation that pituitary disease is accompanied by disturbances in the carbohydrate-tolerance, do not concur with him in his view of the relationship of the disturbance to hyper- or hypo-functioning of the pituitary gland. It must be recollected in this connection, however, that our means of distinguishing between hyper- and hypo-activity of the pituitary gland are rendered very imperfect by the fact that the physical effects of previous hyperactivity of the pituitary body persist, and may in fact constitute the most prominent symptoms, long after the condition has passed into one of deficient activity of the gland.

The possible involvement of the nervous system in the etiology of diabetic conditions was very strikingly brought into prominence by the discovery of Claude Bernard in 1854 that injury of a certain area in the medulla oblongata induced a transitory but severe glycosuria. The particular area concerned lies between the level of the origins of the auditory nerves and the vagi. The **Diabetic Puncture** is most successful in animals that have been well fed with carbohydrates and may fail in ill-nourished animals. The immediate cause of the excretion of sugar which follows this operation is a pronounced **Glucohemia**, the sugar in the blood rising from the normal level of 0.10 or 0.15 per cent.

to 0.3 per cent. or more, and the kidneys simply excrete that proportion of the blood-sugar which constitutes an excess over the normal amount.

The glucohemia which ensues after the diabetic puncture is evidently due to a failure of the normal power of the liver to store up glucose in the form of its anhydride, glycogen. The efficiency of the operation is proportional to the glycogen-content of the liver at the time it is performed, and at the end of the process the liver is found to have been drained of its glycogen-reserves. It appears that the storage-capacity of the liver is subject to control by the nervous system. The afferent path in the reflex arc is contained in the vagi. If the vagus is cut and the peripheral end is stimulated no glycosùria ensues, but if the central end is stimulated a decided discharge of sugar from the liver occurs. The efferent paths lie in the splanchnic nerves, and if these be previously severed the diabetic puncture is without effect.

The greatest advance toward the interpretation of spontaneous diabetes, however, occurred when in 1889 von Mering and Minkowski discovered that extirpation of the **Pancreas** in animals produces a profound glucohemia and glycosuria terminating ultimately in the death of the animal. The effects of this operation have been very exhaustively studied in recent years by F. M. Allen who finds that glucohemia and glycosuria may be induced by partial removal of the pancreas. If nine-tenths of the gland be excised a severe diabetes ensues, but if only a small part of the pancreas, for example one-eighth, be removed, a mild diabetes ensues which is modifiable by diet. Thus if a sufficiency of the pancreas be left *in situ* no glycosuria at all may appear in the urine. If the remnant of gland be larger glycosuria may be absent on a meat-diet or even on a diet containing bread, but glycosuria will ensue if sugars be added to the diet and, once started, may continue on a bread-and-meat diet. In turn, continued glycosuria upon a bread-and-meat diet may culminate in a condition in which glycosuria continues on meat alone, and the experiment terminates fatally.

The interesting observation has been made by Carlson, that if glycosuria be induced in a female animal by depancreatization, and the animal subsequently becomes pregnant, the glycosuria ceases at the time that the pancreas begins to develop in the embryo. It is not certain, however, whether this is due to the mother being enabled to utilize glucose herself through transmission of a pancreatic hormone from the fetus to the maternal circulation, or whether, which is perhaps more probable, the drainage of carbohydrates from the mother by the needs of the fetus deprives her of the excess which she is unable to utilize herself.

In fatal cases of diabetes it has repeatedly been observed that degenerative changes are present in certain elements of the pancreatic tissues, namely the **Islets of Langerhans**, and it is particularly to the removal of these elements that the diabetes following total or partial extirpation of the pancreas is due. Thus injection of paraffin into the ducts arising from the secretory tissues of the pancreas results in

complete atrophy of the secreting epithelium, the Islets of Langerhans alone remaining unimpaired. Under these circumstances no glycosuria occurs, but if this atrophied remainder of the gland be removed typical pancreatic diabetes at once occurs. When the pancreas is only partially removed the overstrain upon the remainder of the tissues leads to their degeneration and the symptoms, possibly slight at first, become progressively more severe. According to Allen, however, if the residue of pancreatic tissue be sufficient and overstrain be avoided by a diet low in carbohydrates and in fats, the incidence of progressive degenerative changes in the residual tissues may be avoided.

The occurrence of spontaneous **Diabetes** in human beings has been recognized from very ancient times, but the actual identification of the sweet constituent of the urine as **Glucose** was not accomplished until 1838. It is characterized, it would appear, almost if not quite invariably by a distinct **Glucohemia**. It is improbable that any cases of spontaneous and persistent glycosuria are due solely to increased permeability of the renal epithelium such as may be brought about experimentally by the administration of phloridzin. The light forms of diabetes resemble alimentary glycosuria except in the fact that the **Assimilation-limit** for carbohydrates is unusually low so that glycosuria recurs whenever a normal abundance of carbohydrate is ingested. In such cases the mere performance of muscular work may arrest the glycosuria. Between this light form of diabetes and the more severe forms every intermediate stage may be observed, and not infrequently the same patient may pass through all degrees of severity of the disease successively. In most severe forms of diabetes sugar continues to be eliminated on a pure protein diet and the urine may contain over ten per cent. of glucose, being usually, but not invariably, dark and discolored from the presence of other abnormal constituents arising from the disordered metabolism.

The sugar which is excreted in the severe forms of diabetes does not arise from carbohydrates in the diet or in the tissues, for not only does it continue on a carbohydrate-free diet, but the quantity excreted *per diem* may be far in excess of the carbohydrates in the food and in the tissues of the body added together. Thus in one experiment upon a depancreatized dog Pflüger found that out of a total excretion of 3097 grams of sugar only 422 grams could possibly be accounted for as arising from carbohydrate reserves of the animal. The difference, namely 2675 grams, must have arisen from some other source. Lüthje even went so far as to feed a depancreatized dog completely upon casein. In eight weeks it excreted nearly 1200 grams of sugar, only a small proportion of which, of course, could have been derived from glycogen in the tissues of the animal. Since the fats, upon a diet such as this, are very quickly used up, we have no alternative but to assume that the sugar was derived in part from the decomposition of proteins and, as a matter of fact, in the severer forms of diabetes there is a decided tendency for the ratio of the sugar

(dextrose) to the nitrogen eliminated in the diet to approach a constant level. This ratio, designated usually by the symbol $\frac{D}{N}$ is regarded by Lusk as affording valuable indication of the severity of the diabetes, for he finds that upon an exclusively fat-and-protein diet the $\frac{D}{N}$ ratio in the severest cases of diabetes approaches a critical value of 3.65 : 1. If the sugar excreted were wholly derived from protein this would mean that from 6.25 grams of protein decomposed in the tissues of the diabetic, 3.65 grams, or 58 per cent. of the weight of the protein, was transformed into glucose. This Lusk believes to be the maximal quantity of carbohydrate which is obtainable from protein, and he illustrates this by reference to the following figures:

Maximum $\frac{D}{N}$ ratios observed in:

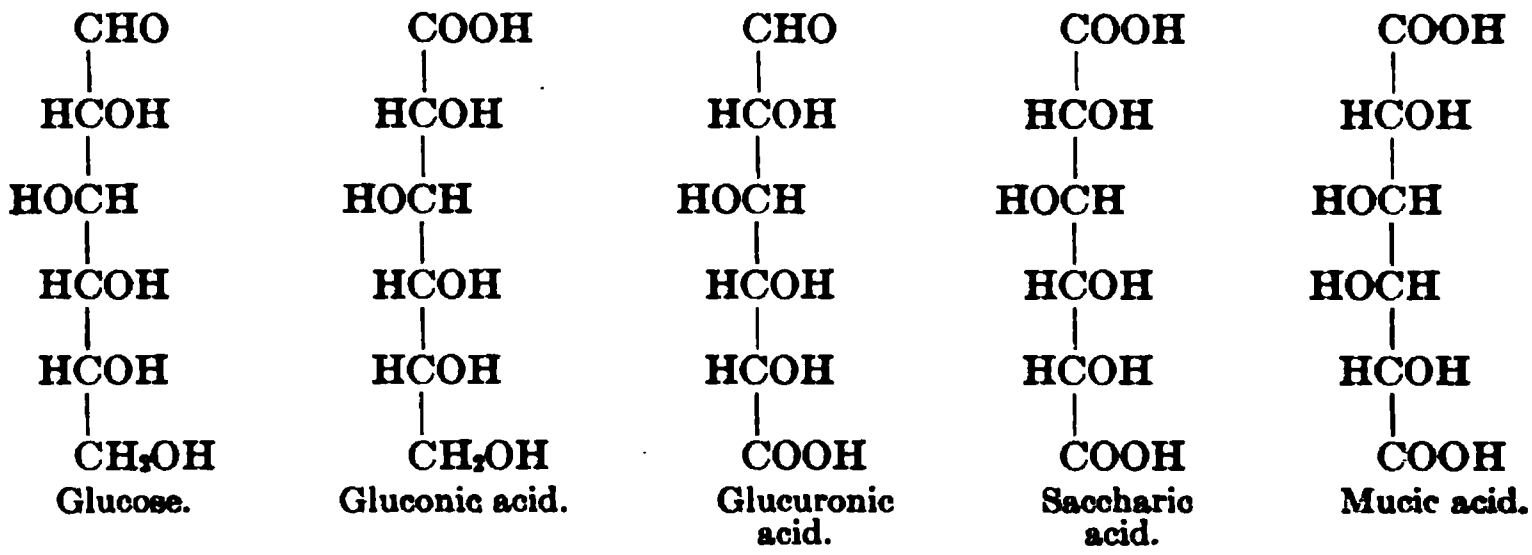
Phloridzin diabetes.		Diabetes mellitus in man.				
In dog. Lusk.	In man. Benedict.	Mandel and Lusk.	Grünwald.	Foster.	Mosenthal.	Joslin.
3.65	3.58	3.60	3.75	3.58	3.75	3.69
3.66	3.82	3.65	3.56	3.38	3.85	3.67
3.62	3.66	3.66	3.70	....	3.44	3.67
3.64	3.68	3.64	3.64	3.48	3.66	3.68

According to Joslin these high ratios, which usually only precede death by a brief interval, are never observed if **Fats** be excluded from the diet, a fact which is a very striking illustration of the determinative part played by fats in the evolution of diabetic symptoms, a part, however, which has only in recent years come to be fully appreciated, thanks to the work of Allen, Joslin, Bloor and other investigators.

The excretion of sugar in diabetes mellitus is not attributable to loss of glycogen storage-capacity on the part of the liver, for even in fatal cases, and after a prolonged excretion of sugar, appreciable quantities of glycogen may still be found in the liver. It is naturally a difficult matter to ascertain whether or not the storage capacity of the liver in diabetics is fully normal, but there can be no question but that the main abnormality of the carbohydrate metabolism in diabetes is essentially a failure to utilize the glucose in the diet. The tissues which are unable to utilize glucose are nevertheless starving for it and every possible mechanism for manufacturing glucose from other foodstuffs, even as we have seen, from proteins, is pressed into service, but the product of these efforts is still glucose and, therefore, worthless or even worse, for it is excreted from the body and involves a corresponding wastage of the fuel-value of the dietary.

The failure of the diabetic to oxidize glucose does not by any means originate in a failure of oxidative powers in general. On the contrary the relationship of the condition to glucose and also as we shall see, to

the fats is highly specific and the oxidation of other and even much more difficultly oxidizable substances may be normal; thus **Lactic Acid, Mannitol** and even **Inosite** or **Benzene** are oxidized just as well by the diabetic as by the normal individual. Even a very slight degree of oxidation of glucose itself suffices to enable the tissues to overcome the obstacle. Thus gluconic acid, glucuronic acid, saccharic acid and mucic acid are all readily oxidized by a diabetic. The relationship of these substances to glucose may be seen from the following formulæ



Even more surprising is the fact that sugars other than glucose may be very much better utilized by a diabetic than glucose itself. **Cane-sugar** is badly tolerated, as might be expected from the fact that it yields glucose on hydrolysis. For the same reason **Maltose**, which yields two molecules of glucose when hydrolyzed, is even less well tolerated by diabetics than cane-sugar. **Lactose** is very badly tolerated probably because it gives rise, on hydrolysis, not only to glucose but also to **Galactose** which is very poorly assimilated by diabetics. **Levulose**, on the contrary is comparatively well assimilated. In many cases it is possible to administer levulose to diabetics without untoward symptoms when similar quantities of glucose would precipitate a profound glycosuria. Depancreatized dogs will store up glycogen on a levulose diet when they cannot do so on a diet containing equal quantities of glucose. For this reason, since levulose is somewhat expensive, it has been proposed to administer **Inulin** to diabetics. Inulin is a polysaccharide of levulose which occurs in the tubers of dahlias, the tuberous artichoke and the sweet potato. It is, however, indigestible by any of the alimentary juices and simply increases the bulk of the feces and provides a culture-medium for intestinal bacteria. The bacteria in the lower intestine certainly attack inulin and the products of their activity may be absorbed or utilized, but these products are not of a carbohydrate nature, for if inulin be administered to an animal with phloridzin glycosuria, no increase of sugar-output is observed. Inulin is therefore of little if any value to a diabetic.

Now it is a very significant fact that when levulose is tolerated by a depancreatized animal, it is converted into glycogen in the liver. This would point, seemingly, to a failure of the liver to convert glucose into glycogen in diabetics, although it is well able to store the glycogen

when it has once been formed. A slight change in the configuration of the molecule of sugar which is absorbed and carried to the liver enables the liver to perform its customary function.

The **Urine** of diabetics has very frequently a pronounced fruity odor, and is usually decidedly acid in reaction. These characteristics of diabetic urine are due to the presence therein of extraordinary amounts of **Aceto-acetic Acid**, CH_3COCH_2COOH, **Acetone**, CH_3COCH_3 and **Hydroxybutyric Acid**, $CH_3CH(OH)CH_2COOH$. These products are all closely related to one another and unquestionably arise from the same source. Thus aceto-acetic acid may be derived from hydroxybutyric acid by oxidation, water being split off, while aceto-acetic acid, with the loss of carbon dioxide, is convertible into acetone. It is probable that hydroxybutyric acid is the parent substance of all the "acetone-bodies" which are found in the urine of diabetics. The production of these substances rapidly and in large amounts, produces the extreme **Acidosis** which is characteristic of the later stages of untreated or improperly treated diabetes, and which culminates in the **Diabetic Coma** or acid-intoxication which formerly was the invariable and still is the very frequent termination of the disease.

The amount of acetone in diabetic urine is comparatively small and it is of minor significance. The aceto-acetic acid may be detected by the deep red color which is communicated to urine containing this substance if **Ferric Chloride** solution be added to it in excess of the amount necessary to precipitate the phosphoric acid as ferric phosphate. It was formerly believed that the acetone bodies in urine were derived from the imperfect oxidation of **Carbohydrates** and that they probably represented intermediate stages in the degradation of carbohydrates to carbon dioxide and water. This view has now been abandoned with the recognition of the fact that **Fats** play a predominant part in the genesis of diabetic **Acidosis**. The very slight change in the glucose molecule which suffices to render it assimilable and utilizable points, in any case, to the improbability that succeeding stages in the oxidation of glucose are exceptionally delayed in the tissues of the diabetic. If oxybutyric acid were in truth an intermediate stage in the oxidation of carbohydrates, as lactic acid, for example is known to be, then the accumulation of this substance in the blood and in the tissues must mean that the subsequent steps of oxidation have become exceptionally difficult. But the very slightest initial oxidation of glucose renders it readily utilizable, so that we must infer that all stages of oxidation succeeding the formation of gluconic or glucuronic acids, for example, are readily performed by the diabetic. Now oxybutyric acid, if it were formed at all from glucose, must succeed the formation of gluconic or glucuronic acids, so that the accumulation of this substance in the tissues of a diabetic evidently cannot be due to the arrested oxidation of carbohydrates. As a matter of fact, Macleod and Pearce have found that the oxidation of glucose in the tissues of depancreatized or even in eviscerated animals is in no way defective, and Meltzer and Kleiner

have shown that a large part of the glucose which circulates in the blood of a diabetic is actually utilized by his tissues.

The examination of the blood in diabetics very frequently reveals, not only glucohemia, but also a pronounced **Lipemia**, which may be so severe as to give to the centrifuged blood-serum a distinctly milky appearance. The following are results obtained by Bloor in estimating the lipoids in normal and in diabetic blood:

	Total fatty acids, grams per 100 c.c.			Lecithin, grams per 100 c.c.			Cholesterol, grams per 100 c.c.		
	Whole blood.	Plasma.	Corpuscles.	Whole blood.	Plasma.	Corpuscles.	Whole blood.	Plasma.	Corpuscles.
Diabetic extremes	.41–.76	.46–.93	.33–.62	.26–.50	.17–.48	.32–.60	.19–.44	.16–.65	.17–.24
Diabetic average (34 analyses)	.52	.59	.43	.36	.30	.46	.29	.36	.20
Normal average (19 analyses)	.37	.39	.34	.30	.21	.42	.22	.23	.20
Normal extremes	.29–.42	.30–.47	.27–.45	.28–.33	.17–.26	.35–.48	.19–.25	.19–.31	.17–.24

It will be observed that the percentage of all the lipoidal constituents of the plasma is much increased in diabetics, while the lipoidal constituents of the corpuscles remain comparatively unaffected. The increase is especially marked in the **Neutral Fats** (estimated as fatty acids) and in the **Cholesterol** fractions. The lecithin or **Phospholipin** fraction increases also but in much less proportion than the others, so that the ratios $\frac{\text{fatty acid}}{\text{lecithin}}$ or $\frac{\text{cholesterol}}{\text{lecithin}}$ are abnormally high in diabetic blood-plasma. For this reason it has been suggested that part at least of the failure of diabetics to utilize fat is due to an inability to convert neutral fatty acids into phospholipins.

The attention of earlier investigators of diabetes was focussed upon the intolerance of these patients for carbohydrates, and the main objective of the physician was to decrease the output of glucose in the urine. Carbohydrates were therefore necessarily excluded from the diet, and to replace the deficient calorific value thus entailed the fats in the diet were not unusually increased. This procedure frequently had the gratifying result, for the time being, of reducing or even eliminating the output of glucose in the urine, but sooner or later the patient, whose condition at first seemed much improved, would again begin to excrete glucose; a severe acidosis developed and the case became hopeless, terminating in diabetic coma.

This result has been duplicated by F. M. Allen in partially depancreatized dogs, and he attributes it to the progressive degeneration of the **Islets of Langerhans** in the residual tissue due to overstrain. As a source of protein he administered beef-lung to the animals, and suet was employed as a means of administering fats. The following is his description of a typical result:

"We may take the customary treatment of moderate diabetes and

illustrate it in dogs. Suppose that suitable operation and overfeeding have produced a condition where there is marked glycosuria on a kilogram of lung, but sugar-freedom on 800 grams of lung, together with a fair state of nutrition and entire absence of ketonuria. Now place the dog on 600 to 800 grams of lung and 100 to 200 grams of suet, according to the classical method. There is no glycosuria, weight is gained, and the condition is splendid for weeks and possibly months. The treatment is highly successful. Closer examination shows the presence of hyperglycemia and slight ketonuria[1] which are usual in the patients of corresponding type. Glycosuria follows, illustrating the spontaneous downward progress which the authorities describe. This is cleared up by a few fast-days on the Naunyn plan, and the diet is again adjusted; it may now be 400 grams of lung and 200 grams of suet. The gain in weight continues as before, with hyperglycemia, ketonuria and subsequent glycosuria. Again the fast days are used and the protein diminished, so that the diet is perhaps 200 grams of lung and 200 grams of suet. The same cycle is repeated. Now the dog is in splendid condition and spirits, the coat sleek, the appearance such that he might create a good impression out walking in the park, only he has a difficulty in remaining sugar-free on even the protein minimum, and the fat may be pushed higher to maintain nutrition against the repeated fast days. If the dog has actually been kept fat, a fasting period about this time may diminish the glycosuria or it may remain high. The previously lively and hungry animal begins to show a curious little mournfulness, and complete repugnance to food. A day or two later, vomiting of clear mucus begins, and the dog drinks and vomits water. The acetone-reaction is heavy; the ferric chloride may be heavy or slight. The alkali-reserve of the blood falls low, and the complete picture of patients who go into fatal acidosis on fasting is reproduced."

As Joslin has pointed out, patients with severe diabetes may struggle on, contending against many complications, and surviving for years on an "atrocious diet," but let a doctor intervene, eliminate carbohydrates from the diet and replace them by an equicalorific allowance of fat, and the patient promptly dies in diabetic coma. The treatment is completely successful, no doubt, in the sense that glucose temporarily disappears from the urine, but the patient nevertheless dies.

Diabetes is, in fact, a multiple metabolic disorder of which the failure to utilize glucose is merely one manifestation which only indirectly induces the fatal outcome. The exclusion of carbohydrates from the diet renders calorific equilibrium and the maintenance of tissue- and body-weight impossible, unless fat be partaken of, not in usual, but even in unusual quantities. The diabetic, however, has a genuine inability to oxidize fats, and intermediate products, of which the leading examples are oxybutyric and aceto-acetic acids, are formed and accumulate in dangerous and ultimately fatal amounts.

[1] "Acetone bodies" in the urine.

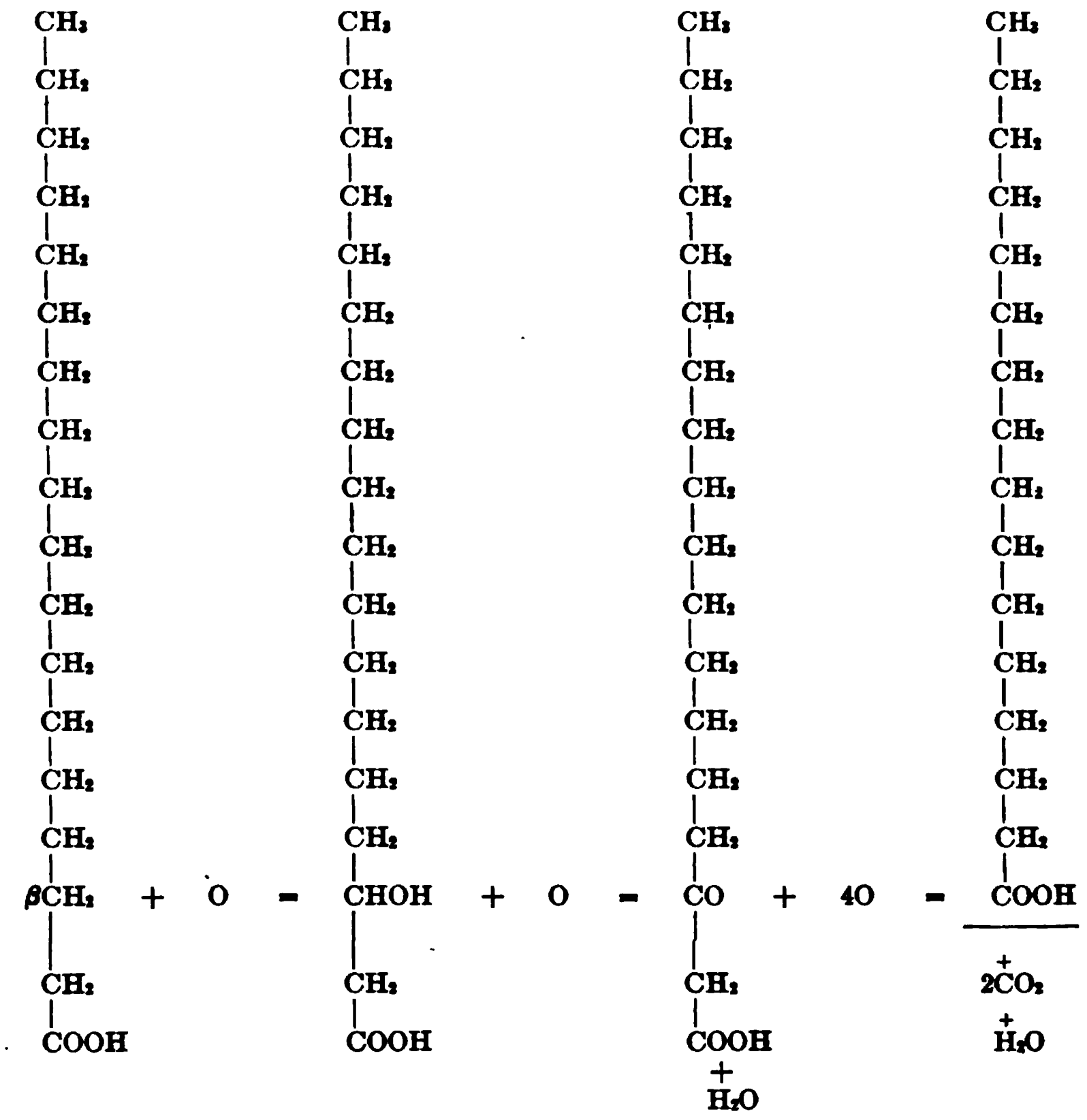
In this dilemma the only feasible procedure is to take advantage of the long-recognized fact that the tissues may be educated by habitude to the proper utilization of carbohydrates, but the slightest overstrain upon the carbohydrate-utilizing mechanism produces a directly contrary result and accelerates the downward course of the diabetic. This is the foundation of "**Allen's Paradoxical Law,**" namely, that "whereas in normal individuals the more sugar is given the more is utilized, the reverse is true in diabetes." The treatment suggested by Allen consists essentially in freeing the urine from glucose by starvation, bearing in mind, however, the fact that starvation increases acidosis and that if the preceding acidosis was high the additional acidosis of too severe or too prolonged starvation may precipitate **Diabetic Coma.** The starvation-period is succeeded by a period in which proteins are admitted to the diet. Carbohydrates are now admitted, at first in very small, and then in gradually increasing amounts, until a tolerance is built up. Fats are admitted last of all, and with great caution, the allowance never being a large one. Patients treated in this way cannot commit dietary indiscretions, but they may maintain a tolerably normal and healthy existence for a number of years. Whether the "expectation of life" of a diabetic may, by a systematic regimen of this kind, be rendered equal to that of a normal individual of like age and antecedents, cannot as yet be stated, for the treatment of diabetes based upon a full realization of the part played by fats in the genesis of fatal symptoms has only recently come into being, and statistics are therefore not available. Furthermore the number of psychological factors which enter into the successful treatment of any chronic disease must be carefully borne in mind in adjudging the statistics when they do become available. The physician may know very well what ought to be done, but in practice he may rarely achieve it. The fluctuating coöperation of attendants, and the fragmentary attention of the busy practitioner to any individual case; the thousand personal details of means, circumstances, behavior, temperament, and metabolism, which render every individual case a separate problem which differs from any other, these factors combine to detract from the success of any method of treatment of a chronic disease-condition, however theoretically perfect the method may chance to be. It is probable, indeed, that to correctly evaluate any method of treatment of a chronic condition we should look to the successful cases rather than to the failures. The ideal means of attaining success would be, of course, to educate the patient to become his own doctor. Unfortunately, however, many patients are unteachable, and most physicians are bad pedagogues.

To revert to the questions of intermediate metabolism which render the phenomenon of diabetes of such exceptional interest to the biological chemist; it appears very probable that **β-Hydroxybutyric Acid** is one of the normal intermediate steps in the oxidation of fats, just as lactic acid is an intermediate step in the oxidation of carbohydrates,

and that in diabetics, through failure of a particular tissue, namely the islets of Langerhans in the pancreas, the further stages of oxidation are hindered, just as, in asphyxia, the oxidations of carbohydrates subsequent to the production of lactic acid are hindered. The appearance of abnormal quantities of **Cholesterol** in the blood of diabetics suggests the possibility that the metabolism of the **Hydroxyaromatic Derivatives** is also disordered in diabetics. The other "acetone-bodies" in diabetic urine are undoubtedly derived from β-hydroxybutyric acid. Thus, if the liver be perfused with blood containing this substance, the blood which issues from the liver contains aceto-acetic acid, and even minced liver will bring about the same transformation.

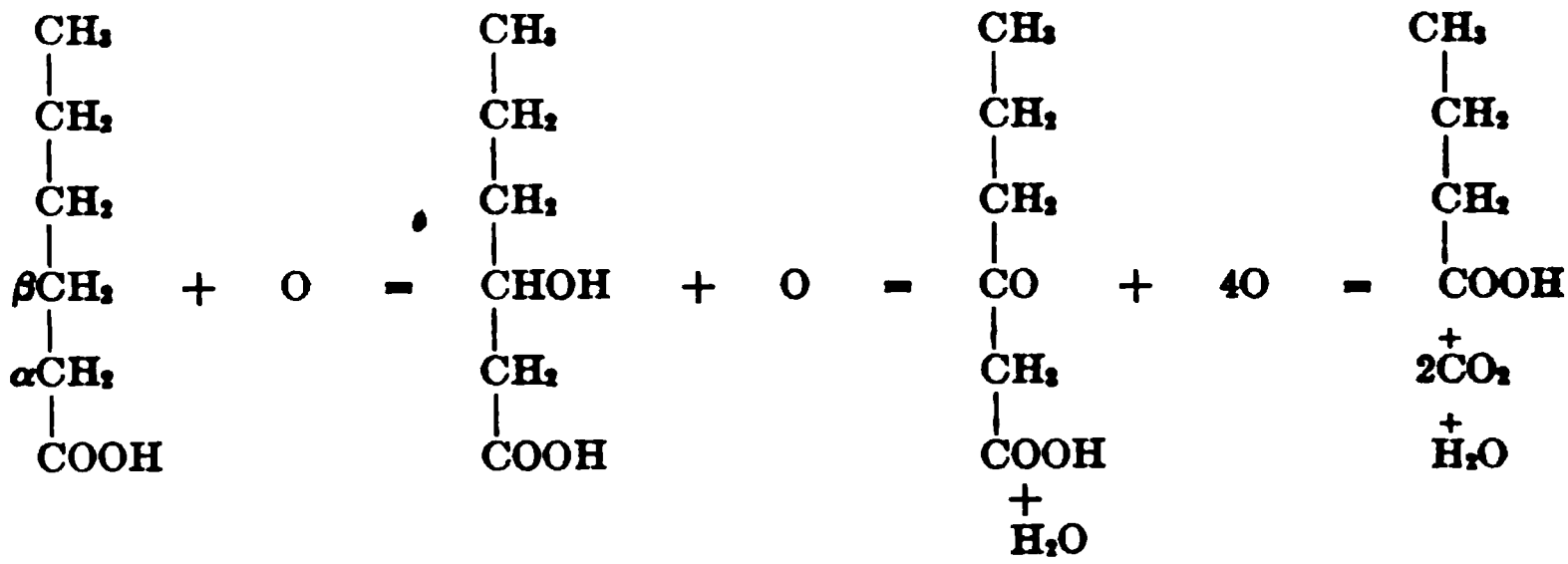
Butyric acid is converted quantitatively by oxidation into β-hydroxybutyric acid, whereas Magnus-Levy has pointed out that 100 grams of **Neutral Fat** made up of tristearin, tripalmitin and triolein can yield a maximum of only 36.2 grams of β-hydroxybutyric acid. Hence cream or **Butter Fat**, with its high content of butyrates, is a much more dangerous source of "acetone bodies" than mutton-fat or bacon-fat or butter-substitutes such as oleomargarine. This fact is illustrated very strikingly in the intolerance which infants frequently display to cream or butter, exhibiting decided symptoms of **Acidosis** when these are administered in what, for other children, would be moderate amounts. These infants not infrequently tolerate a higher fat, or even olive oil, much better than they will tolerate cream or butter.

The oxidation of the fats appears to take place in a series of similar successive steps, the point of attack at each stage in the oxidation being the β-carbon atom, that is, the second carbon atom in the hydrocarbon chain, counting from the carboxyl-group. Thus **Stearic Acid** is converted into **Palmitic Acid** in the following way:



Stearic acid. | Intermediate hydroxy-acid. | Intermediate keto-acid, water. | Palmitic acid, carbon dioxide, water.

In a similar manner palmitic acid yields myristic acid, the next product is lauric acid and this is followed in succession by capric, caprylic and caproic acids. This acid, on oxidation of its β-carbon atom yields butyric acid:



Caproic acid. | Intermediate hydroxy-acid. | Intermediate keto-acid, water. | Butyric acid, carbon dioxide, water.

It is at the next succeeding stage of this process that trouble originates in the diabetic. In normal tissues the intermediate hydroxy- and keto-acids are present only evanescently, being immediately oxidized to the lower acid, carbon dioxide, and water. In the diabetic or in the depancreatized animal there is exceptional difficulty in accomplishing this, it would appear, especially when the stage of butyric acid has been reached and the result is that the partial products of butyric acid oxidation, β-hydroxybutyric acid and aceto-acetic acid are permitted to accumulate:

$$\begin{array}{lcl} \mathrm{CH_3} & & \mathrm{CH_3} \\ | & & | \\ \beta\mathrm{CH_2} & + \mathrm{O} = & \mathrm{CHOH} \\ | & & | \\ \alpha\mathrm{CH_2} & & \mathrm{CH_2} \\ | & & | \\ \mathrm{COOH} & & \mathrm{COOH} \\ \text{Butyric acid.} & & \beta\text{-hydroxybutyric acid.} \end{array} \quad \begin{array}{lcl} & & \mathrm{CH_3} \\ & & | \\ + \mathrm{O} = & & \mathrm{CO} \\ & & | \\ & & \mathrm{CH_2} \\ & & | \\ & & \mathrm{COOH} \\ & & \text{Aceto-acetic acid.} \end{array} \quad + \mathrm{H_2O}$$

If, however, the number of carbon atoms in the original fatty-acid molecule had chanced to be *uneven* instead of even, the final product of this process would have been **Propionic Acid**, CH_3CH_2COOH instead of butyric acid.

Now the important discovery has been made by Ringer, that propionic acid is completely converted into **Glucose** in animal tissues, an intermediate stage in the process being, not improbably, the formation of **β-Lactic Acid**:

$$\begin{array}{lcl} 2\mathrm{CH_3CH_2COOH} & + \mathrm{O_2} = & 2\mathrm{CH_2OH.CH_2COOH} \\ \text{Propionic acid.} & & \beta\text{-lactic acid.} \end{array}$$

He furthermore finds that when fatty acids having an uneven number of carbon atoms are administered they are similarly transformed, in part, into glucose. It happens, however, that the acid radicals of the normal tissue-fats of our dietary always contain even numbers of carbon atoms. The fatty acids possessing uneven numbers of carbon atoms are comparatively rare, and do not occur to any important extent in the fats of the normal dietary, otherwise their administration to diabetics would enable them to transform the residual unoxidized fragment of the fatty molecule into glucose, which is harmless, instead of hydroxybutyric acid which is toxic.

The normal products of the complete oxidation of butyric acid according to the above scheme, would be, successively, **Acetic Acid**, carbon dioxide and water:

$$\begin{array}{lcl} \mathrm{CH_3} & & \mathrm{CH_3} \\ | & & | \\ \beta\mathrm{CH_2} & + \mathrm{O} = & \mathrm{CHOH} \\ | & & | \\ \alpha\mathrm{CH_2} & & \mathrm{CH_2} \\ | & & | \\ \mathrm{COOH} & & \mathrm{COOH} \\ \text{Butyric acid.} & & \beta\text{-hydroxybutyric acid.} \end{array} \quad \begin{array}{lcl} & & \mathrm{CH_3} \\ & & | \\ + \mathrm{O} = & & \mathrm{CO} \\ & & | \\ & & \mathrm{CH_2} \\ & & | \\ & & \mathrm{COOH} \\ & & +\mathrm{H_2O} \\ & & \text{Aceto-acetic acid, water.} \end{array} \quad \begin{array}{lcl} & & \mathrm{CH_3} \\ & & | \\ + 4\mathrm{O} = & & \mathrm{COOH} \\ & & + \\ & & 2\mathrm{CO_2} \\ & & + \\ & & \mathrm{H_2O} \\ & & \text{Acetic acid, carbon dioxide, water.} \end{array}$$

In accordance with this view, Knoop has found that if aromatic derivatives of fatty acids containing an even number of carbon atoms be administered to animals, **Phenyl-acetic Acid** appears in the urine, while if aromatic derivatives of fatty acids containing an odd number of carbon atoms in the molecule be administered, the phenyl-group is split off as **Benzoic Acid** which, as usual, combines with glycocoll in the tissues and appears in the urine as **Hippuric Acid.**

OXIDIZING ENZYMES.

In a variety of animals and plants there are to be found substances which are capable of accelerating certain oxidations. These substances, in the majority of cases, resemble the hydrolyzing enzymes in the minute quantities in which they are effective, and in their instability toward heat. In other cases they are thermostabile and even resist boiling. The discovery of the oxidizing enzymes we owe to the versatile investigator Schönbein (1799–1868), who employed **Guaiacum Tincture** as a means of detecting them. This substance is tinged blue, a coloration due to oxidative changes, by many tissues and tissue-fluids in the presence of peroxides, such as, for example, **Hydrogen Peroxide.** It is found, however, that the oxidizing ferments do not by any means act upon all oxidizable substances equally, on the contrary there is a high degree of **Specificity** in their effects. Thus the enzyme or group of enzymes occurring in the liver and in the spleen which oxidizes **Purines**, converting, for example, **Xanthin** and **Hypoxanthin** into **Uric Acid**, does not attack alcohols, aldehydes or polyphenols. On the other hand the alcohol oxidizing ferment or **Alcoholase** which oxidizes ethyl alcohol to acetic acid, does not attack purines or polyphenols.

The best-studied examples of the oxidizing enzymes are those which are afforded by the **Laccases**, which bring about the hardening of lacquer varnish. A very active enzyme has been prepared from the sap of *Rhus succedanea* by Bertrand, who coagulates the sap with alcohol, redissolves the coagulum in water, and then recoagulates with alcohol. The coagulum is dried *in vacuo* and is then obtained as a white powder which is readily soluble in water, and is characterized by its high content of **Manganese.** The activity of the laccase in oxidizing polyphenols is, in fact, dependent upon the presence of manganese. Thus Bertrand, in studying the action of a similar substance from lucerne with and without the aid of manganous salts, obtained the following results:

Manganous salt alone	0.3 c.c. oxygen absorbed
Laccase from lucerne, alone	0.2 " " "
Laccase plus manganous salt	6.3 " " "

We know that in many cases the oxides of polyvalent metals may act as carriers of oxygen, through the intermediate formation of **Peroxides** which are more active oxidizing agents than free oxygen itself. An

example which is very familiar to biological chemists is that afforded by the action of alkaline copper salts upon glucose. If a limited quantity of **Fehling's Solution** be run into a boiling solution of glucose the solution is decolorized and the red cuprous oxide is precipitated, but upon exposing the mixture in a shallow vessel to the air, the cuprous oxide again takes up oxygen, passes into solution and tinges the fluid blue. If the mixture be now boiled, the cupric hydroxide again parts with its oxygen to the excess of glucose, so that if the process be repeated a sufficient number of times a limited quantity of Fehling's solution will oxidize a relatively unlimited quantity of glucose. This is, in fact, the chief pitfall in the practical employment of Fehling's method of sugar estimation. In a similar manner many other metal oxides are capable of acting as activators or carriers of oxygen.

According to Bach and Chodat the oxidizing enzymes which occur in the majority of living tissues in reality consist of two parts: the one part, the **Oxygenase**, playing the role that cuprous oxide plays in the oxidation of sugar, namely that of a carrier of oxygen, while the other part, the **Peroxidase**, facilitates the transfer of the oxygen from the oxygenase to the material which is undergoing oxidation. **Hydrogen Peroxide** may in many cases take the place of the oxygenase, and hence we obtain the blueing of guaiacum tincture when blood or a tissue-extract together with hydrogen peroxide act upon it. The function of the manganous salt in **Laccase** appears to be associated with the oxygenase fraction, while in many oxidizing ferments found in animal and other plant tissues, **Iron** plays the role which manganese plays in determining the activity of laccase. In this connection it is of great interest to note that **Hemoglobin** is itself an oxygenase, and that, according to Bertrand, the **Benzidine** and **Guaiacum** tests for blood are, in actuality, tests for hemoglobin.

The majority of the oxidizing enzymes appear to be substances of a complex character, in many cases either protein in nature or closely associated with protein. It is probable that the majority of the oxygenases or oxygen-carriers are bodies analogous in complexity to hemoglobin and, like hemoglobin, containing iron or some other polyvalent metal as an integral portion of the molecule. On the other hand Euler and Bolin have shown that the **Laccase** from *Medicago sativa* is a relatively simple substance, being a mixture of the calcium salts of aliphatic hydroxy-acids. A synthetic mixture of the calcium salts of glycollic, citric, malic and mesoxalic acids was found by them to exert the same action in accelerating the oxidation of polyphenols as the natural laccase. This enzyme is, of course, thermostabile.

An important group of oxidizing enzymes is that of the **Tyrosinases** which convert tyrosine into dark-colored substances of complex structure known as **Melanins**, which are probably identical with or closely allied to many naturally-occurring pigments. These enzymes are found in many vegetable tissues and von Fürth has also found them in the tissue-fluids of many insects and in the ink-sac of the cephalopod

Sepia. These oxidases are also able to accomplish the oxidation of other hydroxy-aromatic compounds, such as **Catechol** and **Quinol.**

An enzyme which is often mistakenly regarded as an oxidizing enzyme is the **Catalase** which occurs in nearly all living tissues and which possesses the property of decomposing peroxides, without, however, liberating active oxygen. It is to this enzyme that the frothing of hydrogen peroxide when added to blood or saliva is due. It is in fact a retarder of oxidations and not an accelerator, for it anticipates the action of peroxidase upon peroxides and decomposes them, thus depriving them of ability to transfer oxygen to oxidizable materials. It is probably to be regarded as a controlling agent or check upon overactivity of oxidizing enzymes. It has been shown by Burge that the catalase-content of different tissues varies very greatly, that of the liver being greatly in excess of the catalase-content of muscular tissues. The curious observation has been made by Burnett, however, that if a small proportion of liver-tissue be mixed with muscle-tissue the power of this mixture to decompose hydrogen peroxide is equal to that of an equal weight of pure liver-tissue. This looks either as if catalase really consists, like the oxidases, of two parts, of which only one is contained in considerable amount in muscle-tissue, or else the catalase in muscle-tissue is present therein, not as such, but in the form of a proenzyme or zymogen, which is activated by liver-tissue.

BIOLUMINESCENCE.

The phenomenon of bioluminescence or "phosphorescence" which is displayed by so many organisms, both vegetable and animal, has recently been subjected to very careful study and analysis by N. Harvey. The peculiarity of bioluminescence is the extraordinary intensity of the light which is developed, without any perceptible waste of energy, in the form of heat, an ideal unattainable by any means of illumination at present within our control. The luminescence is dependent upon the occurrence of oxidations, for it disappears when the luminescent system is deprived of oxygen, even when the luminescence is made to occur independently of the life of the organism, as in extracts made from the luminous tissues.

Both Dubois and Harvey, the two leading investigators of this phenomenon, are agreed that the production of luminescence in animal or plant-tissues or tissue-extracts requires the interaction of two substances. The one of these is, according to Harvey, the substance from which the luminescence proceeds, and it is progressively consumed in the process; this he terms the **Photogenin.** The other substance facilitates the oxidation of the photogenin and is termed by Harvey **Photophelein.** The photogenin is colloidal, *i. e.*, does not pass through a dialyzing membrane of parchment, and its light-producing ability is destroyed by heating. The photogenin from the luminous crustacean, *Cypridina,* at all events, appears to be a protein. It is

associated with iron, copper and manganese, but whether the presence of these metals is essential to its luminescence has not yet been ascertained. The proteolytic enzymes destroy its light-producing power. Photophelein, on the other hand, appears to be a substance related to the proteoses or peptones in many of its properties, but it is not digestible by proteolytic enzymes and it is soluble in alcohol.

The separation of photophelein and photogenin from one another may be accomplished by extracting the luminescent animals or organs with hot water. This extracts the photophelein and destroys the photogenin. A solution of photogenin may be prepared by extracting a luminous organ with cold water and allowing the extract to stand until all luminescence has disappeared, when the photophelein has been apparently exhausted. On now mixing these two non-luminous solutions a bright luminescence at once appears.

The actual source of light has been shown by Harvey to be the photogenin, in the following ingenious manner: The light emitted by the Eastern American firefly *Photinus* is orange in color, while that emitted by *Photuris* is greenish-yellow. If, now, photinus photogenin is mixed with photophelein from either *Photinus* or *Photuris* the color of the luminescence is that emitted by *Photinus*, namely orange, and conversely *Photuris* photogenin yields greenish-yellow light whether the source of photophelein be *Photuris* or *Photinus*. Evidently, therefore, the character of the light emitted is determined by the photogenin and not by the photophelein.

The action of photophelein is, to a limited extent, specific. Thus firefly-photophelein will cause emission of light by photogenin derived from other insects, but none from photogenin derived from crustaceans. On the other hand photogenin may be caused to luminesce by many substances which are not of animal and vegetable origin, and particularly by fat-solvents and other **Cytolytic Agents**. Thus luminescence of photogenin may be caused by ether, chloroform, saponins or bile-salts. Harvey believes that these substances promote oxidation of the photogenin by increasing the fineness of the subdivision of the colloidal particles of which it is composed, and thus increasing the area of exposure to oxygen.

The part played by photogenin itself may also be imitated by a variety of reagents. Thus many aldehydes, polyphenols such as pyrogallol, terpenes, waxes, glucose, lecithin, cholesterol, cetyl and myricyl alcohols, tannic and gallic acids, certain peptones and the bile-acids will emit luminescence when treated in certain concentrations with specific oxidizing-agents. **Pyrogallol**, for example, will luminesce when treated with plant **Peroxidases** or with **Hemoglobin** or by certain salts such as potassium permanganate and potassium ferrocyanide, if hydrogen peroxide is also present. For each oxidizer and oxidizable substance there is an optimal concentration above and below which the light-emission diminishes. Thus one-molecular pyrogallol solution will give no light if mixed with $\frac{m}{10}$ potassium ferrocyanide and a little

three per cent. hydrogen peroxide, but $\frac{m}{100}$ or $\frac{m}{1000}$ pyrogallol will give a bright light, while the light from $\frac{m}{100000}$ pyrogallol is only just visible. On the other hand $\frac{m}{2}$ potassium ferrocyanide gives no light with a mixture of $\frac{m}{100}$ pyrogallol and hydrogen peroxide, while $\frac{m}{20}$ potassium ferrocyanide causes bright light-emission.

The phenomenon of bioluminescence therefore depends upon the simultaneous presence, in solution, of a special type of oxidizable substance, and an oxidizing agent which presents some analogies to an oxidase, but is thermostabile and diffusible, and to some extent used up in the reaction which it accelerates.

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CHAPTER XVIII.

PROCESSES INFERRED FROM INDIRECT OBSERVATION; THE ENERGY-TRANSFORMATIONS IN LIVING ORGANISMS.

THE INFLUENCE OF TEMPERATURE UPON LIFE-PROCESSES.

The influence of elevation of temperature upon a chemical reaction may be twofold. If the reaction is at all exo- or endothermic, that is, if any heat is liberated or absorbed during the progress of the reaction, an elevation of temperature will bring about a definite change in **Equilibrium** so that at the conclusion of the reaction the final relative proportion of the various components is altered. On the other hand a rise in temperature always *accelerates* the attainment of equilibrium whatever the station of equilibrium may chance to be. Thus, notwithstanding the fact that the majority of the hydrolyses which occur in living tissues are exothermic, so that a rise in temperature tends to shift the equilibrium in the direction of less complete hydrolysis, yet the rate of **Hydrolysis** being more than proportionately accelerated, enzymatic hydrolyses which are barely perceptible at low temperatures become extremely rapid at the body-temperature of warm-blooded animals.

The effect of temperature upon the velocity of a chemical reaction may be expressed by the equation:

$$\frac{k_1}{k_0} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_1\ T_0}\right)}$$

in which "k_1," and "k_0" signify the velocity-constant at the absolute temperatures "T_1" and "T_0" respectively, "e" is the base of the Napierian logarithms and μ is a constant, differing in different reactions, but almost invariably possessed of such a value that the ratio $\frac{k_1}{k_0}$ exceeds 2 when $T_1 - T_0 = 10°$. The **Temperature-coefficient** of a chemical reaction therefore, or the ratio:

$$\frac{\text{Velocity of the reaction at T} + 10°}{\text{Velocity of the reaction at T}}$$

is in almost every case greater than 2 and may be very greatly in excess of this value.

The behavior of physical, that is to say molecular phenomena rather than atomic, which are affected by temperature, is quite different. The effect of temperature is in these phenomena quantitatively much less than it is in phenomena which arise from chemical transformations. Thus the **Viscosity** of a liquid is diminished by an elevation of temperature, it is true, but the reduction of viscosity which is brought about by a rise of ten degrees in temperature does not exceed about twenty per cent., so that the ratio:

$$\frac{\text{Viscosity at T} + 10^\circ}{\text{Viscosity at T}}$$

is 1.2 or thereabouts. Consequently all the physical phenomena in solutions which are dependent upon the viscosity of the solvent, such as electrical **Conductivity**, and **Diffusion** are affected in a similar degree by elevation of temperature. Those phenomena of which the rate is determined by changes of **Surface-tension** have, in fact, a temperature-coefficient of less than unity, the velocity of changes in capillary tension being actually reduced by elevation of temperature.

One consequence of this decided quantitative difference between the effects of temperature upon chemical and physical phenomena is that we may, with a fair degree of confidence, employ the temperature-coefficient of a complex phenomenon which involves physical as well as chemical changes as a means of gauging the extent to which the velocity of the process is governed by the chemical transformations which it involves. If the pace is set by the rate at which some chemical change transpires, then the rapidity of the process will be at least doubled and not improbably more than doubled by a rise of ten degrees in temperature. But if the chemical transformations are subordinate to some physical process and must await its development before they can proceed, or if they are simply consequent upon physical changes such as electrolysis, or alterations in surface-tension, then the pace of the whole process will be set by this physical event and the temperature-coefficient of the process may be expected to be less than 2 or even very considerably less than 2.

We have already seen that the various enzymatic hydrolyses which occur in the digestion of the foodstuffs yield temperature-coefficients which lie between 2 and 4; all of them exceeding 2 at temperatures which are not too far above the temperature of the warm-blooded animals. The temperature-coefficient of enzymatic processes necessarily declines very rapidly at temperatures which are much in excess of 40°, because at these temperatures the acceleration of the auto-destruction of the **Enzyme** itself is so great that its loss of activity more than compensates for the gain in the velocity of the hydrolysis which the residual undestroyed enzyme is able to bring about. We have, in fact, to deal with the resultant of two opposed processes both of which are accelerated by elevation of temperature. At lower temperatures

the acceleration of hydrolysis is the predominant result of raising the temperature, but at higher temperatures, destruction of the enzyme becomes the controlling factor. The temperature-coefficient for enzyme destruction is exceptionally high, so that the rate of autodestruction may be imperceptible between 30° and 40° and extremely rapid at temperatures lying between 40° and 50°.

Even in a single uncomplicated chemical transformation the temperature-coefficient is not constant, for, reverting to the equation:

$$\frac{k_1}{k_0} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_1\ T_0}\right)}$$

we see that the temperature-coefficient for 10° temperature-interval is given by:

$$\frac{k_1}{k_0} = e^{\frac{\mu}{2}\left(\frac{10}{T_1\ T_0}\right)}$$

it is therefore not independent of the absolute magnitude of the temperature employed; in fact the temperature-coefficient must invariably decrease as the temperature rises. Assuming a value of μ (=13, 200) which would yield a coefficient of 2 between the temperatures of 30° and 40°, the following table shows the coefficients which might be anticipated at other temperatures:

Temperature interval.	Temperature-coefficient.
0° to 10°	2.34
10° to 20°	2.22
20° to 30°	2.11
30° to 40°	2.00
40° to 50°	1.92

The reduction of the coefficient for enzyme reactions at temperatures above 40° is, however, much more extreme than could be accounted for in this fashion, the coefficient ultimately falling to zero at the thermal limit for the activity of the enzyme.

The actual phenomena of life are almost invariably of a mixed character, involving physical as well as chemical processes and changes, and we may inquire through the investigation of their temperature-coefficients whether the physical or the chemical factors predominate in determining the rate of performance; whether the chemical transformations, in other words, are consequent upon preceding physical changes or whether, on the contrary, the physical modifications of protoplasm await and are the resultant of the chemical transformations which accompany the performance of vital activities.

The first investigator to apply this criterion to the study of life-phenomena was Cohen, who in 1892 pointed out that the previous

measurements by Clausen of the rate of production of **Carbon Dioxide** by germinating seeds showed that this process is approximately doubled in velocity by an elevation of 10° in temperature until an upper limit somewhat exceeding 40° is attained, when the rate of the tissue-respiration falls off owing to heat-injury. This method of inquiry was extended to animal tissues by C. D. Snyder, who investigated the influence of temperature upon the **Rate of the Heart-beat** in the isolated heart of the Pacific terrapin, *Clemmys marmorata*. The following are illustrative results:

Time of exposure to the temperature minutes.	Number of heart-beats per minute.					
	Heart 1.	Heart 2.	Heart 3.	Heart 4.	Heart 5.	Heart 6.
	T. = 10°.		T. = 20°.		T. = 30°.	
5	9.5	9.5	21.5	21.0	48	48
10	7.0	9.0	21.0	24.0	48	44
15	6.7	8.7	19.0	18.0	48	40
20	7.0	8.2	19.0	16.5	41	
30	7.0	7.0	16.0	14.0		
40	6.5	7.9	15.5	15.5		
50	6.5	7.9	13.5	16.0		
60	6.2	7.4	13.0	15.0		

It is evident that the rate of the beat is approximately doubled for each 10° rise in temperature. From the data quoted and others obtained by Snyder the following average coefficients may be computed:

HEART-BEAT OF CLEMMYS MARMORATA

Temperature-interval.	Temperature-coefficient for 10° intervals.
10° to 20°	2.3
20° to 30°	2.2
30° to 37°	1.6

at temperatures exceeding 37° the rate of the beat, instead of increasing, diminished until the heart came to a standstill owing to irreparable heat-injury. These experiments were subsequently repeated upon the isolated heart of another species of terrapin, *Emys europea*, by Galeotti and Piccinini, and by Snyder upon the isolated heart of the frog, and by Kanitz upon the isolated mammalian heart.

The heart *in situ* is, however, considerably modified in its behavior and particularly in the rate of beat by the nervous control to which it is subjected. The study of the heart-beat in the intact animal therefore involves more numerous and more complex factors than that of the beat of the excised heart. Nevertheless in this case also the rate of the beat is primarily determined by the velocity of underlying chemical changes. Thus in the minute transparent fresh-water crustacean, *Ceriodaphnia*, the heart can be viewed through the body-wall of the

animal and the beats counted at a variety of temperatures. The following are illustrative of the results obtained by this method:

Temperature interval.	Temperature-coefficient.
11° to 21°	2.76
15° to 25°	2.24
17° to 27°	2.05
19° to 29°	2.06
21° to 31°	1.14

at a temperature slightly above 31° the heart-beat ceases and the organism dies.

In the case of the crustacean *Limulus* the **Heart-ganglion** can be heated or subjected to other manipulation without directly involving the heart-muscle itself, and Carlson has found that by heating the ganglion alone the heart-beat is accelerated, the unusually high temperature-coefficient of 4 being obtained.

On the other hand, in the **Embryonic Heart,** in which the mechanism of nervous control is probably not yet established, the rate of the heart-beat is similarly affected by temperature, being doubled or trebled by a rise of ten degrees. The following are results obtained by Loeb and Ewald, employing the embryos, still enclosed within the egg, of the marine fish *Fundulus:*

Temperature.	Time required for 19 heart-beats in the embryo; seconds.
30°	6.25
25°	8.5
20°	11.5
15°	19.0
10°	32.5
5°	61.0
10°	33.5
15°	18.8
20°	12.0
25°	10.0
30°	6.0

It is evident, therefore, that both the muscular and nervous mechanisms involved in the regulation of the rate of the heart-beat are primarily conditioned, as to their velocity, by underlying chemical transformations.

Loeb and Ewald have drawn attention to the fact that in *Fundulus* embryos the rate of the heart-beat is almost the same in all the embryos exposed to the same temperature, provided they still remain enclosed within the egg. This is because of the elimination of all secondary disturbing factors. As soon as the embryos begin to move, this equality disappears, because the motility of different embryos differs and the products discharged from the contracting muscles influence the rate of the heart-beat. In man and in other higher animals, the number of the disturbing factors, while the heart remains *in situ*, are so great that no uniformity of rate at any given temperature can be expected. "Differences in emotions or the internal secretions following

the emotions, differences in metabolism, differences in the use of narcotics or drugs, and differences in activity are only some of the number of variables which enter" (Loeb). Hence the attempt to compute the temperature-coefficient of the heart-beat *in situ* in man from the acceleration of the beat in **Fevers** is illogical, and we find, as a matter of fact, a great deal of discordancy in the values computed from data of this kind, coefficients varying from 1.8 to 5 having been reported.

The **Respiratory Rhythm** is even more susceptible to modification by sensory stimuli, muscular exertion and so forth, than the cardiac rhythm, and consequently the coefficients which have been observed for the rate of the respiratory movement at different temperatures are not of so uniform a character as those which are cited above. Nevertheless the influence of temperature upon the **Respiratory Center** is extremely striking. It has long been a familiar fact that warming the blood in the carotid artery, by causing it to flow through a heated tube, results in a marked acceleration of the respiratory rhythm, and in frogs it has been shown that the direct application of heat to the floor of the fourth ventricle leads to a very decided increase in the rapidity of respiratory movements. It is an extremely interesting fact that the effect of temperature upon the respiratory rhythm of cold-blooded animals is very much greater at a low oxygen-tension than at a high, possibly because when the oxygen-tension is low and the consumption of oxygen by all of the tissues is accelerated by an elevation of temperature, the effect of the temperature elevation itself is aided by the stimulation of the respiratory center which lack of oxygen indirectly entails, while when oxygen is abundant there is sufficient for the needs of all the tissues even at high temperatures, and the secondary stimulation of the center does not occur.

The influence of temperature upon the velocity of "**Basal Metabolism**" or tissue-respiration can only be studied in cold-blooded animals under conditions which exclude muscular movement, which would, of course, introduce irregular fluctuations in the rate of consumption of oxygen. This problem has been approached by Krogh in several ingenious ways. One method was to employ the pupæ of insects in which tissue-respiration is of course maintained but muscular movement is arrested. The following were results obtained with the pupæ of the mealworm, *Tenebrio molitor:*

Temperature.	Oxygen-consumption per kilogram-hour.	Temperature-coefficient per 10° C.
10.°	43.5	5.7
15.°	104.	3.2
20.°	185.	2.6
25.°	300.	2.2
30.°	445.	2.2
32.5°	529.	

It will be observed that the temperature-coefficient is very high at low temperatures and falls rapidly as the temperature rises. A similar

characteristic distinguished the temperature-coefficient of the time consumed in the pupal stage of development:

Temperature.	Hours spent in pupal condition.	Temperature-coefficient per 10° C.
13.45°	1116.	
15.55°	742.	6.2
17.0°	593.	
18.8°	439.6	4.9
20.9°	320.	
23.65°	234.1	
27.25°	172.5	2.6
32.7°	137.9	
32.95°	134.25	1.5

We may infer that the time spent in the pupal stage depends upon the extent of tissue-oxidation which has occurred.

By employing curarized frogs and decerebrated turtles Krogh was also enabled to investigate the effect of temperature upon the **Tissue-respiration** of these animals. The values of the coefficients obtained lay between 2 and 4, but the values were not found to be so greatly affected by the position on the temperature-scale of the temperature-range employed as in the case of the insect-larvæ.

The influence of temperature upon the **Rate of Development** of organisms is again of a similar character. Thus Hertwig has investigated the influence of temperature upon the time taken to reach seven different arbitrarily chosen stages of development of the larvæ of a frog, *Rana fusca*. The following were the results obtained:

Temperature-interval.	Temperature-coefficient for 10°.						
	Stage I.	Stage II.	Stage III.	Stage IV.	Stage V.	Stage VI.	Stage VII.
2.5° to 6°	10.	13.	15.	14.			
6.° to 15°	2.6	2.6	2.5	2.6	3.1	3.5	4.5
10.° to 20°	2.9	3.3	3.2	2.9	3.5	3.4	3.3
20.° to 14°	1.5	1.4	2.0	2.0	2.0	2.0	1.8

We have seen that the rate of development in the pupal stage of insects and the rapidity of their basal metabolism are very similarly influenced by temperature, so that we may infer with probability that oxidations determine the duration of this period of development. This is not the case in the earliest stages of development, however, for Loeb and Wasteneys have investigated the influence of temperature upon the time which elapses between insemination and the first cell-division in sea-urchin eggs and have compared with this the effect of the same temperatures upon the oxygen-consumption of the eggs. The two sets of temperature-coefficients are unmistakably of the magnitude of the coefficients of chemical reactions, but they are very diversely affected by alteration of the position of the temperature-range, as the following figures show:

Temperature-interval.	Temperature-coefficient of rate of segmentation in:		Temperature-coefficient of rate of oxidation in:
	Strongylocentrotus.	*Arbacia.*	*Arbacia.*
3° to 13°	3.91	...	2.18
4° to 14°	3.88	...	...
5° to 15°	3.52	...	2.16
7° to 17°	3.27	7.3	2.00
8° to 18°	...	6.0	...
9° to 19°	2.04	4.7	...
10° to 20°	1.90	3.8	2.17
11° to 21°	...	3.3	...
12° to 22°	1.74	3.1	...
13° to 23°	...	2.8	2.45
15° to 25°	...	2.5	2.24
16° to 26°	...	2.6	...
17.5° to 27.5°	...	2.2	2.00
20° to 30°	...	1.7	1.96

Not only oxidations, therefore, but some other chemical factors even more susceptible to temperature change are involved in determining the rapidity of the cell-divisions in the early stages of development.

Other forms of growth, for example **Regeneration**, as A. R. Moore has shown, are also affected by temperature to the extent characteristic of chemical reactions.

In all of the life-processes hitherto mentioned the general order of magnitude of the temperature-coefficients has been the same. When, however, we come to study the temperature-coefficients for the **Duration of Life** we meet with a startling disparity of quantitative effects, for whereas, for example, it takes a rise of nearly ten degrees to double the rate of the heart-beat, or the rate of respiratory movements or the rate of cell-division or regeneration or tissue-oxidations, yet a temperature-elevation of merely one degree, as J. Loeb has shown, serves to halve the duration of life of fertilized or unfertilized eggs of the sea-urchin, and lowering of the temperature by ten degrees prolongs the life of the organism 2^{10}, that is to say over a thousandfold. The temperature-coefficient of the processes underlying the thermal death of the cells is therefore, no less than 1000. A. R. Moore has investigated the influence of various temperatures upon the duration of life of a hydroid, *Tubularia crocea*, judging viability by the retention of the power of regeneration. The following are illustrative results:

Temperature.	Duration of life.	Coefficient for temperature interval of: 1°.	10°.
25°	55 to 60 hrs.		
26°	25 to 30 "	2.0	
27°	15 to 18 "	1.7	
28°	7 to 8 "	2.2	3900
29°	130 to 140 mins.	3.3	
30°	50 to 60 "	2.4	
31°	30 to 40 "	1.6	
32°	14 to 15 "	2.4	
33°	7 to 8 "	1.9	485
34°	3 to 4 "	2.1	
35°	2 to 3 "	1.4	
36°	1½ to 2 "	1.5	

It is obvious that here we are dealing with a phenomenon of quite a distinct nature from the other phenomena of life which we have hitherto being considering, and the question immediately suggests itself whether any clue exists as to the origin of this remarkable susceptibility to temperature. Now on comparing the temperature-coefficients of various reactions involving **Enzymes**, one group stands out from all the rest by reason of the extraordinary magnitude of the temperature-coefficients, and that is the group afforded by the **Auto-destruction** which various enzymes undergo in solution. The following data are cited after Arrhenius:

Nature of process.	Numerical value of μ.
Hydrolysis of sugar by acids	25,600
" " invertase	9,080
Saponification of ethyl acetate by NaOH	11,150
" triacetin by lipase	16,700
" cottonseed oil by lipase	7,540
Digestion of gelatin by pepsin	10,750
" " trypsin	10,570
" egg-white by pepsin	15,570
Coagulation of milk by rennet	20,650
Spontaneous destruction of trypsin in solution	62,034
" " pepsin in solution	75,600
" " rennet in solution	90,000
" " vibriolysin in solution	128,000
" " tetanolysin in solution	162,000
" " hemolysin in solution	198,500

The relationship between the value of μ in the equation:

$$\frac{k_1}{k_0} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_1\ T_0}\right)}$$

and the temperature-coefficient for the ten-degree interval between 20° and 30° C. is shown in the following table:

A temperature-coefficient of	Corresponding to the value of
2	13,200
10	44,000
100	88,000
1,000	132,000
10,000	176,000

It is evident therefore that the temperature-coefficient of the duration of life corresponds not at all with that of enzymatic hydrolyses, but it is, on the other hand, of precisely the order of magnitude encountered in the autodestruction of enzymes or of specific antibodies. It is to the destruction of enzymes, consequently, that we may attribute the thermal death of organisms excepting in those cases, as in spores of seeds, in which the essential tissue-enzymes are thermostabile and the temperatures required to kill the tissue are those which

suffice to coagulate **Proteins.** For example, Goodspeed, who investigated the thermal death of barley-seeds, exposed them to temperatures ranging from 55° to 70° C. and obtained a temperature-coefficient of 10 for the duration of life, a coefficient which is very close to the value 8 obtained by Chick and Martin in estimating influence of temperatures of similar magnitude upon the rate of coagulation of **Hemoglobin.**

The remarkable disparity between the effects of temperature upon the **Life-duration** and the **Development** of organisms has been applied by Loeb to the explanation of what would otherwise be an exceedingly puzzling fact, namely the extraordinary density of the population of the polar seas. In his account of the Valdivia expedition, Chun[1] calls especial attention to the quantitative difference in the surface fauna and flora of polar and temperate or tropical regions: "In the icy water of the Antarctic, the temperature of which is below 0° C, we find an astonishingly rich animal and plant- life. The same condition with which we are familiar in the Arctic seas is repeated here, namely, that the quantity of plankton material exceeds that of the temperate and warm seas." And again, in describing the pelagic fauna in the region of the Kerguelen Islands he states: "The ocean is alive with transparent jellyfish, Ctenophores (*Bolina* and *Callianira*) and of Siphonophore colonies of the genus *Agalina*."

This observation, which has been repeatedly made by Arctic and Antarctic travellers, would appear paradoxical in consideration of the effect of temperature upon development, for the rate of development of organisms is, as we have seen, halved or even reduced to a greater extent by a drop of 10° C. in temperature. When, however, we reflect that the duration of life of these slowly developing organisms is prolonged a thousandfold, the density of the polar population becomes explicable, for the net result of these opposed effects would be a great increase in the number of surviving individuals and in the number of successive generations simultaneously inhabiting the cold waters.

The temperature-coefficient of the life-processes which we have hitherto considered have all been of such a magnitude as to clearly invite the supposition that the velocities of the phenomena are determined by the rate at which underlying chemical transformations occur. We now come to a life-phenomenon of peculiar character in which the testimony of the temperature-coefficient is far from being so unequivocal, namely the **Conduction of Stimuli** along the fibers of a motor-nerve.

The influence of temperature upon the rate of conduction of the nervous impulse was first investigated by S. S. Maxwell, who employed for this purpose the pedal nerve of a large slug, *Ariolimax columbianus*. This nerve was selected on account of its considerable length and the slowness of the propagation of the impulse permitting a much greater exactitude of measurement than is possible in the shorter and more

[1] Cited after J. Loeb: The Mechanistic Conception of Life.

rapidly conducting sciatic nerves of a frog. The following table summarizes the results:

Temperature-interval.	Temperature-coefficient.
−0.5° to 9.5°	2.14
0.° to 10.°	1.79
1.° to 11.°	1.98
3.° to 13.°	2.07
5.° to 15.°	1.29
6.° to 16.°	1.57
9.° to 19.°	1.32
11.° to 21.°	1.47
11.5° to 21.5°	1.54
12.5° to 22.5°	1.67
13.° to 23.°	1.65
14.° to 24.°	1.32
16.° to 26.°	1.81

It will be seen that almost the only coefficients approaching the numerical value of 2 are those obtained at the lowest temperature-ranges,[1] nor is this a peculiarity of the type of nerve-fiber employed by Maxwell, for the later investigations of Lucas and Gantor on the transmission of impulses in the motor-nerves of frogs bear similar testimony. The following are the results obtained by Gantor:

	Temperature-coefficient for 10° interval. Experimental series number.					
Temperature.	1	2	3	4	5	Average.
0.	..	2.35	1.86	...	...	...
2.5	..	2.28	1.77	...	...	...
5.	2.03	1.79	1.79	1.97	2.09	1.87
7.5	1.82	1.64	1.65	1.95	1.51	1.77
10.	1.79	1.60	1.57	1.95	1.59	1.71
12.5	1.66	1.53	1.62	1.81	1.75	1.64
15.	1.59	1.48	1.55	1.77	1.67	1.61
17.5	..	..	1.50	1.68	...	...
20.	..	..	1.47	1.61	...	...

These coefficients are intermediate in value between those usually obtained in physical phenomena and those which may characterize chemical transformations. We are therefore led to suspect that physical events play a large part in determining the rate of transmission of nervous impulses. This view is rendered the more probable by the historical difficulty which has been encountered in demonstrating the existence of any metabolic changes in nerve-fibers or their enhancement by stimulation, and while the recent results of Tashiro demonstrate a minute evolution of carbon dioxide from excised nerves, it cannot be regarded as proved that this metabolic activity is very closely associated with the conduction of the stimulus. It may, rather, be concerned with the maintenance of the nutrition or repair of the nerve, and the inability of nerve-fibers to display fatigue on repeated stimulation lends strong encouragement to this view, for evidently no material

[1] A small number of coefficients exceeding 2 are attributed by Maxwell to experimental errors and are not included in the above averages.

is used up in consequence of their excitation. On the other hand, Carlson's experiments with the heart ganglion of *Limulus* and the above-cited experiments on the effects of heating the respiratory center show that chemical changes play a predominant role in the activities of **Nerve Cells**, and, as a matter of fact, the consumption of oxygen by the brain is very large, and it is the first tissue to suffer from lack of oxygen, indicating a very high level of metabolic activity in the *cellular* elements of the nervous system. The conducting fibers and the nerve-cells from which they issue stand therefore in sharp contrast to one another in respect to the metabolic foundations of their functional activity, and we are thus led to recall the fundamental difference between their susceptibilities to the various classes of chemical stimulants to which reference has been made in a previous chapter. Nerve-fibers are powerfully stimulated by salts which precipitate calcium, nerve-cells are insensitive to these reagents. Nerve-cells are stimulated by a variety of specific substances, by polyphenols and by **Creatine**, for example, to which nerve-fibers are indifferent.

The phenomena of **Muscular Contraction** and the change which transforms the nervous impulse into a muscular stimulus at the myoneural junctions are, it would appear, conditioned in their speed by underlying chemical reactions. Thus Burnett has determined the influence of temperature upon the **Latent Period** of indirect muscular stimulation (*i. e.*, through the intermediation of a motor-nerve) and finds that the period consumed in the transformation of the nervous into the muscular stimulus is halved or even more reduced by a rise of ten degrees in the temperature. Similarly the changes involved in the stimulation of **Sensory Nerve Endings** are determined by chemical factors, since T. E. Moore has shown that the temperature-coefficient of the reaction to cutaneous stimulation by heat is of the chemical magnitude. Corresponding with these facts we find that nerve-endings readily undergo fatigue. We have seen that the rate of the heart-beat is doubled or more than doubled by a rise of 10° and the same thing has been found to be true for other rhythmic muscular contractions. The rate of conduction of the **Action-current in Muscles**, however, appears, from the investigations of Lucas, to be a process analogous to the conduction of a nervous impulse, comparatively little affected by temperature (coefficient from 1.45 to 1.65).

It is a general characteristic of **Photochemical Reactions**, and a peculiarity which distinguishes them from all other types of chemical transformation, that they are practically unaffected by temperature, the temperature-coefficients being usually unity, and at any rate not in excess of the magnitudes commonly obtained in purely physical phenomena. This being the case it is a very significant fact that the temperature-coefficients of the phenomena induced by light in living organisms are usually high and distinctly of the order indicating the involvement of chemical reactions of the ordinary type. Thus the phototropic bending induced by light in *Avena sativa* has been shown

by de Vries to be increased from three to five times in velocity by a rise of 10° in temperature. Still more remarkable is the fact that the **Assimilation of Carbon Dioxide** by green plants in sunlight which underlies the **Photosynthesis of Carbohydrates** is also doubled or more than doubled by a rise of ten degrees in temperature; the following results are compiled from the measurements of Gabrielle Matthæi, the absorption measured being that of leaves of *Prunus laurocerasus* exposed to gaslight of constant intensity:

Temperature.	Carbon dioxide assimilated.	Temperature-coefficient per 10° C.
−6°	0.2	28.7
0°	1.75	2.40
10°	4.2	2.12
20°	8.9	1.76
30°	15.7	1.81
37°	23.8	0.23
40.5°	14.9	

Heat-injury already appears at 30°, but below this temperature the coefficients clearly indicate that the rate of assimilation is not determined by the photochemical process but by a reaction of the ordinary type. These results may be interpreted by supposing that the photochemical reaction (transformation of CO_2 and H_2O into formaldehyde) is retarded by its product, and that the speed of photosynthesis is therefore determined by the rate at which this product is removed by a secondary reaction (condensation of formaldehyde into glucose).

THE INFLUENCE OF LIGHT UPON LIFE-PROCESSES.

Photosensitive Substances are of very widespread occurrence in living tissues. This is evidenced by the fact that the effects of light upon organisms are not by any means confined to the specialized cells which comprise the visual organs in the higher *metazoa*. The synthesis of **Carbohydrates** in plants is brought about by the action of sunlight upon vegetable tissues which contain **Chlorophyll** or some analogous pigment and are exposed to an atmosphere containing carbon dioxide, but quite independently of this, light additionally exerts an effect upon the protoplasm of the cells of most plants, leading to a bending of sessile forms or an actual migration of motile forms toward or away from the source of light, a phenomenon known as **Phototropism** or **Heliotropism.** This phenomenon is also very generally displayed by animals, and the investigations of J. Loeb have demonstrated that the mechanism of heliotropism in animals and in plants is essentially the same, nor is it invariably associated, even in animals, with the possession of specific light-sensitive organs. For many of the unicellular forms of life sunlight is very definitely toxic, and this is true not only for pigmented but also for colorless cells. The most toxic portion of the spectrum lies in the ultraviolet region, a fact which bacteriologists have attempted

to utilize for the sterilization of water and milk; a difficulty is created, however, by the deficient power of ultraviolet light to penetrate liquids, which necessitates the exposure of only thin layers to the toxic rays.

The region of the spectrum which is most efficient in causing heliotropic movements or curvature varies in different species of animals and plants. Among the plants the rays of the blue end of the spectrum are usually the most efficient. Thus Blaauw has determined the duration of exposure to light derived from various parts of the carbon arc which is necessary to induce heliotropic curvature in the seedlings of *Avena*. The following are his results:

Duration of illumination in seconds.	Position of the rays in the spectrum.
6300	534 $\mu\mu$
1200	510 "
120	499 "
15	491 "
5	487 "
4	478 "
3	...
4	466 "
6	448 "

The maximum effect is therefore obtained between 478 $\mu\mu$ and 466 $\mu\mu$, that is, in the blue region of the spectrum. Among animal forms Loeb and Wasteneys have found that *Eudendrium* and *Arenicola* are similarly affected chiefly by the blue rays, while other animals, for example the crustaceans and insects are primarily affected by the rays lying on the border of the green and yellow. Even closely allied forms may, however, differ very decidedly in the part of the spectrum which is most efficient in eliciting heliotropic curvature. Thus Loeb and Wasteneys have observed that the green flagellate *Euglena viridis* is most affected by the blue rays lying between $\lambda = 470$ and 480 $\mu\mu$, while the closely allied flagellate *Chalmydomonas pisiformis* is especially sensitive to the yellow-green rays in the neighborhood of $\lambda = 534$ $\mu\mu$.

That the foundation of these light effects resides in a **Photochemical Reaction** which is induced within the organism is shown by the applicability of the **Bunsen-Roscoe Law**, which is generally characteristic of photochemical reactions and applies, for example, to the blackening of a sensitized photographic plate by exposure to light. This may be enunciated as follows: The chemical effect induced by light is proportional to the product of the intensity multiplied by the duration of illumination, or in symbols:

$$E = Kit$$

where E is the extent of photochemical transformation, i the intensity of the light, and t the duration of the illumination and K a proportionality-factor which is constant for the particular photochemical transformation under consideration.

The validity of the Bunsen-Roscoe law in the heliotropism of organisms has been established in a variety of investigations. Thus Blaauw has determined the time required to produce heliotropic curvature in the seedlings of *Avena sativa* by varying intensities of illumination with the following results:

Candle-meters.	Duration of illumination.	Candle-meters × seconds.
0.00017	43 hours	26.3
0.000439	13 "	20.6
0.000609	10 "	21.9
0.000855	6 "	18.6
0.001769	3 "	19.1
0.002706	100 minutes	16.2
0.004773	60 "	17.2
0.01018	30 "	18.3
0.01640	20 "	19.7
0.0249	15 "	22.4
0.0498	8 "	23.9
0.0898	4 "	21.6
0.6156	40 seconds	24.8
1.0998	25 "	27.5
3.0281	8 "	24.2
5.456	4 "	21.8
8.453	2 "	16.9
18.94	1 "	18.9
45.05	2/5 "	18.0
308.7	2/25 "	24.7
511.4	1/25 "	20.5
1255.	1/55 "	22.8
1902.	1/100 "	19.0
7905.	1/400 "	19.8
13094.	1/800 "	16.4
26520.	1/1000 "	26.5

It will be seen that the product of the intensity into the duration of the illumination approaches the constant value of 20, and indeed, when one considers the very great range of intensities employed and the inherent variability of living material, the degree of constancy observed is really astonishing. A remarkable instance of the applicability of this law to the **Heliotropism** of animals is afforded by the experiments of Loeb and Wasteneys, upon the polyps of *Eudendrium*. These hydranths are exceedingly variable in their response to light and it was accordingly necessary to make a great number of measurements and treat the results statistically. The polyps were exposed to three different intensities of light, a light of definite strength being stationed at three different distances, namely 25 cm., 37.5 cm. and 50 cm. from the organisms and they were exposed to the light for such periods as to render the product i × t a constant. Under these circumstances it was found that some among any group of polyps underwent heliotropic curvature, while others did not. The percentage of bent polyps was determined in each case, and if the Bunsen-Roscoe law were valid it is evident that these percentages should be the same for all three intensities of light, *i. e.*, the percentages undergoing bending at the distances 25, 37.5 and 50 cm. from the light should be the same or 1 : 1 : 1. The actual ratios were determined for each of the possible

pairs of distances and they differed more or less from the ideal value of unity, but the average of a large number of these ratios differed from unity only by an amount commensurable with the probable error of the average. The following are their results, the values enclosed in brackets being rejected by Chauvenet's criterion for the rejection of extreme variates.[1]

Times of exposure in minutes.			Ratio of percentage of hydranths bending toward light.		
25 cm.	37.5 cm.	50 cm.	25 cm.; 37.5 cm.	25 cm.; 50 cm.	37.5 cm.; 50 cm.
15	....	60	....	1.50	
20	....	80	....	1.30	
10	22.5	40	1.20	(3.08)	(2.56)
10	22.5	40	0.94	1.47	1.55
10	22.5	40	1.57	(2.30)	(2.43)
10	22.5	40	1.43	1.04	0.94
10	22.5	40	0.76	1.09	1.47
10	22.5	40	1.05	1.13	0.90
					0.96
10	22.5	40	1.15	....	0.99
7	15.75	28	0.84	0.62	0.74
7	15.75	28	1.70	0.49	0.58
7	15.75	28	0.85	1.25	1.35
7	15.75	28	(2.09)	0.99	1.08
7	15.75	28	1.14	1.15	0.55
7	15.75	28	0.44	0.92	0.44
7	15.75	28	1.52	0.80	0.61
7	15.74	28	0.59	0.36	0.70
7	15.75	28	0.48	1.07	0.31
7	15.75	28	1.00	0.48	1.80
7	15.75	28	0.69	1.09	0.81
7	15.75	28	1.26	0.85	1.09
7	15.75	28	0.86	1.38	0.85
7	15.75	28	0.70	1.07	1.59
7	15.75	28	....	0.77	1.25
7	15.75	28	....	0.60	
Average*			1.01	0.97	0.98
Probable error*			±0.05	±0.04	±0.06

* The averages and probable errors given are those recalculated by the authors since the original article was published.

It is a general law of **Photochemical Action** that only those rays are effective which are absorbed by the system in which the reaction occurs. Visible light-rays are not, as a general rule, selectively absorbed by protoplasm and hence their action is usually confined to or exerted reflexly through specialized pigmented areas which constitute the receptive elements of optical sense-organs. White light which is not toxic for the majority of tissues may be rendered toxic, as L. Loeb has shown, by impregnating the tissue with certain dyes, particularly **Eosin**, which in such cases acts as the photochemical absorbent or sensitizer. **Ultraviolet Light**, however, is universally toxic even for

[1] W. Chauvenet: A Manual of Spherical and Practical Astronomy, Philadelphia, 1891, vol. 2, p. 558.

colorless organisms, and since this toxicity presumably depends upon and is attributable to photochemical reactions, the question presents itself: To which constituent of the protoplasm are we to attribute the selective absorption of these rays which we may presume to be the necessary precedent to their photochemical activity?

It was pointed out over forty years ago by Soret that the majority of proteins exhibit a well-marked absorption-band in the ultraviolet part of the spectrum. In seeking for the origin of this absorption-band Soret found that it is especially well exhibited by solutions of **Tyrosine** and therefore referred it to the tyrosine radical in the protein molecule. These observations have been extended by Kober, who has carried out a spectrographic examination of solutions of the various **Amino-acids** which are the end-results of protein hydrolysis and of certain **Polypeptides**. Kober has confirmed the existence of an absorption-band in the ultra-violet in solutions of tyrosine, and finds that a similar band is exhibited by solutions of **Phenylalanine**. The other amino-acid constitutents of the protein molecule exhibit only general, *i. e.*, non-selective absorption in the ultraviolet spectrum.

The possibility is thus indicated that the tyrosine and phenylalanine radicals of the proteins constitute the optical sensitizers which render living cells susceptible to the toxic action of ultraviolet light. If this were the case, then passage of the light through solutions of proteins or the aromatic amino-acids should, by absorption of the toxic ray, to a greater or less extent, deprive the light of its toxicity for protoplasm. This possibility has been investigated by Harris and Hoyt, who have found that the passage of ultraviolet light through protein or peptone solutions partially detoxicates it, while passage through solutions of **Cystine, Tyrosine** or **Amino-benzoic Acid** has a remarkable effect in shielding the organisms from injury. Other dissolved substances such as sugar, urea, alanine, glycocoll, etc., were found to be devoid of protective power. **Leucine** undergoes decomposition when exposed to ultraviolet light and it exerts a certain measure of protection. The following are illustrative results, the light from a Cooper-Hewitt ultraviolet light being passed through the solution contained in a quartz beaker before reaching the organisms (*Paramœcia*) suspended in distilled water below the beaker:

Solution.	Average determination-period, seconds.
Water	130
1.0 per cent. alanine	130
1.0 per cent. glycocoll	130
1.0 per cent. aspartic acid	130
1.0 per cent. glutamic acid	135
1.0 per cent. leucine	250
0.5 per cent. tyrosine	420
1.0 per cent. amino-benzoic acid	2400
0.5 per cent. NaOH	150
1.0 per cent. NaOH	170
1.0 per cent. glutamic acid in 1 per cent. NaOH	200
1.0 per cent. cystine in 0.5 per cent. NaOH.	1200
1.0 per cent. tyrosine in 0.2 per cent. NaOH	Unaffected after 40 minutes' exposure.

The absorption of ultraviolet rays by tyrosine has been found by Kober to be markedly increased by an alkaline reaction and, as the above results show, the detoxication of the ultraviolet light by tyrosine solutions is also very greatly increased by an alkaline reaction.

The results of Harris and Hoyt are thus in harmony with the view that the susceptibility of protoplasm to ultraviolet light is conditioned by the selective absorption and consequent "activation" of the toxic rays by the aromatic amino-acid radicals of the proteins. These results have a practical as well as a theoretical bearing, for they imply that fluids containing proteins would be much more difficult to sterilize with ultraviolet light than water, owing to the protective action of the proteins in the fluid through which the light has to pass before it impinges upon the protoplasm of the infecting organisms.

THE STORAGE OF POTENTIAL ENERGY: THE PHOTOSYNTHESIS OF CARBOHYDRATES.

The leaves and other chlorophyll-containing organs of green plants absorb **Carbon Dioxide** from the atmosphere and simultaneously liberate an equal volume of oxygen. The carbon which is thus retained is built up into the tissues or reserve-materials of the plant, appearing chiefly in the form of **Carbohydrates** which accumulate very rapidly during active assimilation.

The process of carbon dioxide assimilation by green plants takes place only in the light and in the presence of **Chlorophyll** or related pigments. Within certain limits the rate of absorption is proportionate to the intensity of the illumination of the leaf, and to the percentage of carbon dioxide in the atmosphere. Not all parts of the spectrum are equally efficient in promoting this process, the red rays between B and C causing the most rapid assimilation while the activity of the rays between D and E on the Frauenhofer scale is a minimum, and there is a second maximum in the violet, beyond F. This was first shown in a most ingenious manner by Engelmann. The **Aërobic Bacteria** require the presence of free oxygen to display motility. If some green algæ and aërobic bacteria be imprisoned together under an air-tight cover-glass and kept in the dark, the free oxygen is soon consumed and the bacteria become motionless. If the cell is now exposed to light the algæ decompose carbon dioxide, setting free oxygen, and the bacteria become motile again. Exposure of the cell in different parts of the spectrum yielded the above-quoted results.

The assimilation of carbon dioxide by green plants is the foundation of the existence, not only of the plants themselves, but of the animal world. The radiant energy of the sun which is in this manner stored up in the tissues of the green plants, reappears at the other extremity of the life-process as the heat or muscular energy and mechanical work of an animal. A very striking peculiarity of living material also originates in this process, for while the components of protoplasm are,

as a rule optically active, *i. e.*, rotate the plane of polarized light to the right or left, the products of laboratory-syntheses and those substances in nature which have never passed through the life-cycle (and some of those which have done so) are optically inactive. It is true that we can decompose a racemic and optically inactive mixture into optically active parts by utilizing the selective enzymatic activities of **Yeasts**, or, as Pasteur did, we may sort out large crystals by hand into two kinds possessing equal and opposite rotatory powers, but it will be observed that all of these processes involve the intrusion of a living agent. According to the view of Byk, optical activity originated in the earth through the circular polarization of light which occurs when light is reflected from the surface of the sea. If, on the other hand, we revert to Arrhenius' theory of the origin of life upon the earth, we may suppose that optical activity was transmitted to this planet by **Bacterial Spores** floating in interstellar space. However this may be, the phenomenon of optical activity is at present a distinguishing characteristic of the components of living matter, and it originates in the very first step in the life-cycle, for the carbohydrates which result from the photosynthetic activities of plants are optically asymmetrical.

Notwithstanding the fact that the immediate connection between the assimilation of carbon dioxide by green plants and the appearance of carbohydrates has long been understood, the intermediate products which are formed in the process; the various stages which link the absorption of carbon dioxide to the appearance of starch or sugars in the tissues, have long been sought for in vain. The classical theory, proposed by Baeyer in 1870, is that the carbon dioxide is first reduced to **Formaldehyde**

$$CO_2 + H_2O \rightarrow HCHO + O_2$$

and that the formaldehyde which is thus formed is subsequently condensed to a hexose:

$$6HCHO = C_6H_{12}O_6$$

If this view is correct then we should expect to find formaldehyde among the constituents of the green plants when engaged in active assimilation. Very many attempts have been made to establish the presence of formaldehyde in the tissues of plants and they cannot yet be said to have yielded any very definite information. Several exceptional difficulties attach to this investigation. In the first place it is certain that if formaldehyde occurs in green leaves at all it is never present except in very minute amounts. Indeed it is essential that this should be so, because formaldehyde is a very powerful protoplasmic poison and the accumulation of any amount in excess of a minute trace would result in the complete arrest of protoplasmic activities. Thus *Elodea canadensis* is cited as a form which is exceedingly resistant to the toxic action of formaldehyde, yet it will only withstand a 0.001 per cent. solution.

We must expect to find formaldehyde in vegetable tissues, if it occurs therein at all, therefore, only in minute traces. Now although we possess very sensitive reagents for aldehydes, yet these do not as a general rule exclude the possibility of much more complex aldehydes than formaldehyde being present and yielding the reaction. Even **Proteins** and many other tissue-constituents will yield reactions indicative of an aldehyde-grouping. This would not perhaps constitute an insurmountable difficulty if it were not for the fact, as we have seen, that the aldehyde we are seeking to identify is at the most only present in minute traces.

Another way of attacking the problem might appear to be feasible, namely that of extracting **Chlorophyll** from green plants and utilizing its light-activating properties to bring about the synthesis of formaldehyde in laboratory-glassware, apart from the complications and secondary reactions which attend the process in living tissues. Numerous attempts to accomplish this have failed. According to Usher and Priestley, however, the source of failure has resided in the employment of comparatively thick layers of the chlorophyll solution. If we blow carbon dioxide through a test-tube or flask filled with chlorophyll and exposed to light, we cannot expect to observe much photosynthesis, because the most superficial layers of the chlorophyll solution will absorb all of the active light-rays and transmit to the underlying solution only those which are chemically inactive. In the living plant the chlorophyll is disposed quite differently. Here we observe that pigment is confined to exceedingly thin layers at the surfaces of a series of bodies known as the **Chloroplasts** in which active photosynthesis can be shown to be proceeding during illumination. Usher and Priestley have sought to imitate this architecture of the photosynthetic apparatus, by painting the surfaces of plates of gelatin with a thin layer of chlorophyll and then blowing carbon dioxide over them and exposing them to light. Under these conditions they state that a comparatively rapid disengagement of oxygen occurs, the surface film becoming wrinkled and distorted by the accumulation of bubbles of oxygen below it, while very evident quantities of formaldehyde are found in the underlying gelatin. The accumulation of formaldehyde in this case, as contrasted with its evanescence in the tissues of plants they refer to the absence of the enzymes necessary to accomplish the removal of the formaldehyde by condensation, which, in the plants, are present in the underlying substance of the chloroplasts. Against this experiment it has been urged by several investigators that the presence of formaldehyde in gelatin jellies is very difficult to establish, since most samples of gelatin themselves yield a very pronounced aldehyde-reaction. Usher and Priestley, however, state that the gelatin which they employed was free from aldehydes. On the other hand the synthesis of formaldehyde and other products from carbon dioxide and water has frequently been accomplished without the intermediation of chlorophyll by the use of the silent electric discharge, and by exposure

to ultraviolet light or to sunlight in solutions containing salts of **Uranium.**

We have seen that the rate of assimilation of **Carbon Dioxide** is governed, not primarily by the velocity of the photochemical reaction, but by the velocity of a subsequent reaction which removes its products. This is shown by the fact that the temperature-coefficient of carbon-dioxide assimilation is of the usual chemical magnitude and not unity, as would be the case in a purely photochemical process. If the product of the photochemical reaction is in truth formaldehyde, as Baeyer's hypothesis assumes, then its accumulation would very evidently be injurious and we can readily understand how its removal, which presumably does not require the agency of illumination, would be an essential condition of the continuance of the reaction and would "set the pace" of the whole process. It is not certain, however, at exactly what stage of carbohydrate-synthesis the necessity for light ceases. Thus W. Loeb has obtained not only the formation but also the partial polymerization of formaldehyde with the silent electric discharge. The fact that starch-formation will go on in tubers and other plant-tissues which are not exposed to the light throws no light upon this question, for the starch in these instances is not formed from formaldehyde but from hexoses or other comparatively complex carbon compounds.

In regard to the nature of the earliest carbohydrate to arise in photosynthesis the most natural supposition would appear to be the formation of **Glucose**

$$6HCHO = C_6H_{12}O_6$$

or some other hexose, since this synthesis has actually been performed in the laboratory. As a matter of fact, however, there is much evidence tending to show that the first carbohydrate to be produced in photosynthesis is actually **Cane-sugar** (sucrose). This view, which was first put forward by Brown and Morris, has received very strong support from the investigations of Parkin. This observer employed for his experiments the leaves of the snowdrop, *Galanthus nivalis*, which are peculiar in that they do not form **Starch** during photosynthesis, so that the analyses of sugar-content are not complicated by the possible presence of sugars, **Maltose** or **Glucose**, derived from the hydrolysis of starch. As a matter of fact it was found by Parkin that the leaves of the snowdrop contain only three carbohydrates, namely **Sucrose, Fructose** (levulose) and **Glucose.** Of these the percentages of hexose remain very constant throughout any given twenty-four hours, not increasing during the illumination of the day, nor decreasing during the night, while the percentage of sucrose rapidly increases during the day and decreases decidedly at night. Moreover the proportion of sucrose to the other sugars is greatest at the apical portions of leaves where assimilation is most active, and decreases toward the base. Two interpretations of this result, however, may

be advanced. The cane-sugar may be in truth the first sugar to be synthesized, or, on the other hand, glucose may be the first sugar formed, levulose arising from it by a transformation which can be accomplished in laboratory-glassware, and the constancy of the hexose percentage may merely mean that hexose in excess of this amount is condensed to cane-sugar as rapidly as it is formed. It should be mentioned, however, that the formation of levulose from glucose by alkalies in laboratory-glassware is accompanied by the simultaneous formation of **Mannose**, which sugar is absent from foliage-leaves. On the other hand no laboratory-method of directly deriving cane-sugar from formaldehyde has yet been discovered.

THE CONVERSION OF CHEMICAL INTO MECHANICAL ENERGY: THE CHEMICAL MECHANICS OF MUSCULAR CONTRACTION.

We have seen that upon a normal mixed diet the necessary energy for the performance of muscular work is derived from the oxidation of **Carbohydrates** and that the final products of this oxidation are carbon dioxide and water, an intermediate stage of the combustion being the formation of **Lactic Acid.** So much we can ascertain by methods of direct analysis. If we desire, however, to complete the story of the energy-cycle which begins with photosynthesis in the plant, and culminates in the release of heat and mechanical work by the animal, purely analytical methods will not suffice and we are impelled to seek additional information by the method of inference from indirect observation.

Our object is to ascertain the nature of the chemical machine which transforms the potential energy of carbohydrates into muscular work and heat. This problem divides itself into two parts, namely the question of the nature of the process of combustion and the question of the means of transforming the energy which combustion releases into mechanical work.

In respect to the first of these questions, it has long been a familiar fact that when a muscle is repeatedly stimulated, either directly or indirectly through its motor-nerve, the first few contractions gradually and with considerable regularity increase in height until they reach a maximum for a given strength of stimulus. This phenomenon to which the name of "treppe" or the **"Staircase Phenomenon"** was given by Bowditch, has been the subject of considerable investigation and conjecture. Of a similar nature is the phenomenon of **"Summation of Stimuli,"** whereby a stimulus of strength insufficient to give rise to a response when it is first applied, may be made, by repetition, to elicit a response.

It is to Waller that we owe the suggestion that the "staircase" is, in reality, due to the increased production of carbon dioxide by the contractile or conducting tissue. He observed that small amounts of carbon dioxide augment the electrical response of nerve-fibers to

stimulation and that a short tetanization of the nerve produced a precisely similar augmentation. Lee has extended this idea to muscular tissues and he has pointed out that the action of the products of muscular activity upon the performance of muscular work is two-fold producing in moderate quantities or for a short time a marked increase in the irritability and working-power of the muscle, while in larger quantities or after a longer period of action they produce a marked depression or "**Fatigue**" of the muscle, ending by totally preventing the further release of muscular energy. The nature of the products which bring about these results has been established by Lee, who has found that perfusion of a muscle with a dilute solution of **Lactic Acid** or an acid phosphate increases its irritability and power to do work, while more concentrated solutions of the same substances diminish and finally abolish its irritability and contractility. Both of these substances are known, by direct estimation, to accumulate in a muscle which is doing work.

If we consider a muscle which is being tetanized by rapidly repeated stimuli, it is evident that the rate at which the muscle is doing work may be regarded as an expression of the rate at which the underlying chemical changes are taking place. During the initial or rising part of the curve of tetanus which is nearly always to be observed, the velocity of the underlying chemical changes must be increasing. During the period of maximal contraction while the recording-lever remains at a constant level it is evident that the rate of doing work and therefore the velocity of the underlying chemical change are practically constant, during the third, or descending part of the curve the velocity of the chemical changes is evidently decreasing. Similar considerations apply, of course, when the muscle, instead of being stimulated at extremely small intervals, is being stimulated at longer intervals.

The chemical changes which underlie and determine muscular contraction are of such a character, therefore, that one or more of the products which result, first accelerate and later retard the process. We are familiar with many chemical reactions of this type; they are reactions in which one of the products acts as a catalyzer to the process and are therefore designated **Autocatalyzed Reactions**. Thus in the hydrolysis of **Cane-sugar** by neutral boiling water small quantities of mucic acid are developed which greatly accelerate the inversion. The hydrolysis of **Methyl Acetate** by water results in the liberation of acetic acid which very greatly accelerates the hydrolysis. The hydrolysis of the **Ricinoleic Acid** in pulverized castor-oil beans proceeds at first very slowly, and then with great rapidity, the acid which is first liberated enhancing the activity of the lipase in the macerated tissues. Instances of autocatalytic oxidations are afforded by the spontaneous oxidation of many **Metals** and organic compounds in the presence of oxygen at atmospheric temperature and pressure. It has long been observed that in the spontaneous oxidation of these substances they acquire the power of inducing oxidations in other substances which are not spontaneously

oxidizable, and it has been shown that this action is due to the formation of peroxides which catalyze oxidations, including the oxidation of the spontaneously oxidizable material itself. In the case of metals the process is ultimately brought to a close by the thickness of the covering of oxide which excludes the air. To preserve metals from spontaneous oxidation, therefore, one of two methods should be adopted: Either they should constantly be kept clean and polished to avoid the accumulation of catalyzers, or they should be allowed to become so completely tarnished that air can no longer penetrate to the underlying metal. The intermediate policy of sporadic infrequent polishing leads to maximal loss of the metal by oxidation.

Very many instances of autocatalysis are afforded by the spontaneous oxidation of fats and oils, and particularly by the oxidation of the "**Drying Oils**" which are employed in paints and varnishes.

It is a general characteristic of the processes of **Autocatalysis** that they begin relatively slowly, progressively increase in velocity to a maximum, and then fall off in velocity again until the reaction finally ceases. The cessation of the reaction may be due to the exhaustion of the **Substrate** or material undergoing transformation, as for example in the hydrolysis of cane-sugar, or it may be due to the back-pressure of the accumulated products, as in the case of the hydrolysis of methyl acetate. In general the autocatalyzers, like other catalysts, accelerate the attainment of equilibrium from either direction.

The underlying combustion which releases the heat and mechanical energy of muscular contraction is therefore an example of a large class of chemical transformations which produce their own catalyzers. Of the various stages of the process only a few are known, but among the known products lactic acid and carbon dioxide are capable of identification as direct or indirect catalyzers of the combustion.

Our knowledge of the second phase of the problem which is presented by the genesis of muscular work and heat is still more fragmentary and much more conjectural. No machines of the ordinary type with the details of which we are familiar, such as those which operate by gaseous or liquid pressures and mechanical thrusts, will even approximate in characteristics and behavior to the motile mechanisms of living protoplasm. The low and only very slightly fluctuating heat of combustion precludes any explanation attributable to alternate expansions and contractions due to heating and cooling. Engelmann, indeed, has proposed such an explanation, based upon the supposition that intense heating of minute particles in the muscle-substance may occur in a number of circumscribed foci. He has pointed out that a number of **Doubly Refracting Substances**, such as catgut or India-rubber, have the unusual property of contracting when they are heated, and he assumes that the heat-energy of combustion in muscular tissue is directly transformed into mechanical work by transient intense heating of localized doubly refracting elements. Many objections have been urged against this hypothesis and they appear in our present state of knowledge to

be insurmountable. The objection, for instance, that living matter is destroyed at the height of temperature required was met by Engelmann by supposing that the elements so heated only form a very small proportion of the whole contractile tissue. If this be so, then they cannot be the doubly refractile elements which we perceive under the microscope, for these form a very large proportion of the whole. The foundation of Engelmann's analogy between muscular tissue and catgut or caoutchouc therefore falls to the ground. Furthermore even the small proportion of the structural elements of muscular tissue which Engelmann assumes to be subjected to heating, having been destroyed thereby, would have to be decomposed and the products excreted. Muscular work should therefore consume muscle-tissue and the nitrogenous excretion should increase. This, however, on a normal mixed diet, does not occur. Again, the intense local heating which Engelmann assumes implies difficulty in the distribution and dissipation of the heat which results in muscular combustion, yet the swift relaxation which succeeds normal muscular contraction implies just the reverse. A direct transformation of heat into work through the agency of expansion or contraction is therefore an improbable explanation of muscular contraction.

Other observers have sought to attribute the phenomena of muscular contraction to the **Swelling** or shrinkages of semisolid elements through the imbibition or giving up of water, as a jelly absorbs water from or parts with it to the surrounding medium. The known processes of this kind are, however, relatively slow and gradual in development, whereas muscular contraction and relaxation may in the muscles of the insect's wing alternate no less frequently than 300 times per second.

The only physical displacements which are capable at the same time of such rapid alternation, of the performance of so much mechanical work in a non-rigid system, and of transforming so large a proportion of energy into mechanical work as a living muscle, are the displacements which result from changes in **Surface-tension**. These are excessively rapid because the forces involved are of great magnitude and the frictional resistances which oppose them may be, under favorable conditions, very small. The amount of energy stored up in a fluid surface is very great and the release of this energy by chemical or resultant electrical changes affecting the tension of a large surface would suffice to permit the performance of a large quantity of mechanical work. The maximal attainable **Efficiency** of a surface-tension engine as Brunner and Wolf have shown, is fifty per cent., *i. e.*, the heat absorbed in extending the surface of water is equivalent to one-half of the mechanical work done in producing the surface-extension. This is also the maximal efficiency which has ever been observed in the performance of muscular work.

The earliest theory to regard a muscle as a surface-tension engine was that proposed by Imbert who assumed that the individual **Fibrils**

which are demonstrable in muscular tissue are long thin cylinders which are maintained in a condition of elongation by passive stretching. Under the influence of increase of surface-tension at the surface of contact of these tubules and their fluid contents Imbert assumes that the tubules become more spherical and therefore shorter, the simultaneous shortening and swelling of a number of these elements leading to the contraction of the muscle. According to this hypothesis the work performed in muscular contraction is derived from changes in the surface energy of the fluid contained in the tubules. Bernstein, rather drastically assuming certain magnitudes for the tension and alterations thereof at the surfaces of these tubules, inferred that if the tubules consist of the visible muscle-fibrils then the surface afforded is not sufficient to account, on Imbert's hypothesis, for the amount of work performed in contraction. If, however, we suppose that the fibrils are broken up into a number of separate elements, for example into rows of ellipsoids which become spherical when the tension of their surfaces increases, then the surface presented would be sufficient to account for the observed release of mechanical energy. Now recent investigations by Schäfer, McDougall and others on the details of the microscopic structure of muscle, have revealed the presence in the fibril, not exactly of the structure imagined by Bernstein, but one that for the purposes of Imbert's hypothesis is precisely equivalent to it. Schäfer describes the contractile elements of the muscle-fiber as fine columns or **Sarcostyles** which are divided into segments or **Sarcomeres** by thin transverse discs, known as **Krause's Membranes.** Each sarcomere contains a relatively opaque portion, the **Sarcous Element,** while those portions adjacent to Krause's membrane are relatively transparent and seen to consist of a fluid material. The sarcous element itself is double and, if stretched, the two portions separate at a line which runs transversely across the opaque portion of the sarcomere (Hensen's line). On contraction the sarcous elements become shorter and thicker, absorbing the fluid which constitutes the **Hyaloplasm** or intervening transparent area between the sarcous elements and Krause's membrane. We may therefore picture the muscle-fibril as consisting of a series of discs formed by minute tubules packed together and communicating with spaces separating the discs and filled with fluid (Fig. 25). Evidently such a structure as this conforms to every requirement imposed by Bernstein upon Imbert's hypothesis, and it is an exceedingly significant fact that the details of the structure which we have outlined become clearer and more elaborate as we successively pass from the relatively sluggish and inert smooth muscles, or the striated muscles of amphibia, to the muscles of insects with their lightning-like rapidity of contraction and enormous power of performing work. This fact alone prevents us from entertaining any doubt that this elaborate structure is an essential part of the muscular mechanism, and the salient characteristic of this structure is the enormous surface of contact which it brings about between the fluid and the semisolid

elements of the tissue. We may therefore with considerable confidence infer that muscular tissue is *a surface-tension engine* which converts the energy released by the combustion of carbohydrates into heat and mechanical work.

Several mechanisms are imaginable whereby the chemical changes which accompany muscular work might bring about alterations of surface tension at interfaces within the tissue. The **Heat of Combustion** of carbohydrates must of itself contribute to affect the tension and the changes of **Electrical Potential** which also accompany muscular contraction would likewise, as is shown by the analogy of Lipmann's

FIG. 25.—Wave of contraction passing over a leg-muscle fiber of water-beetle. (After Schäfer.)

capillary electrometer, affect the tension of interfaces in the tissue. In this connection one fact should be very particularly noted, and that is that either of these factors, and whether they are determinative or not they must contribute in some measure to the outcome, would lead, not to an *increase* of superficial tension, as imagined by Imbert and Bernstein, but to a *decrease*. We are therefore led to inquire whether, after all, the alteration in form of the sarcous elements in contraction may not be due to a decrease rather than to an increase in interfacial tension, for otherwise the thermal and electrical changes which accompany muscular contraction must actually diminish and inhibit contraction and conflict with the main objective of the whole process.

A mechanism whereby reduction of interfacial tension might bring about the shortening of sarcous elements is depicted in the accompanying figure (Fig. 26). If we suppose the sarcous element to consist of an elastic tube dipping into and filled by the fluid hyaloplasm, then the tension of this fluid, pulling upon the elastic tubule will draw the walls inward and hence stretch them longitudinally. This condition of balanced tensions, capillary and elastic, may be supposed to be the normal resting state of muscle. If, now by heat, an electrical potential, or other means, the inward pull upon the tubule is released, this will have just the same effect that internal pressure would have upon an initially unstretched elastic tube—it will expand and shorten, as a hose expands and shortens when water under a head or pressure is suddenly injected into it. Thus the ends of the sarcous elements will approach and, the total capacity of each element being increased, fluid

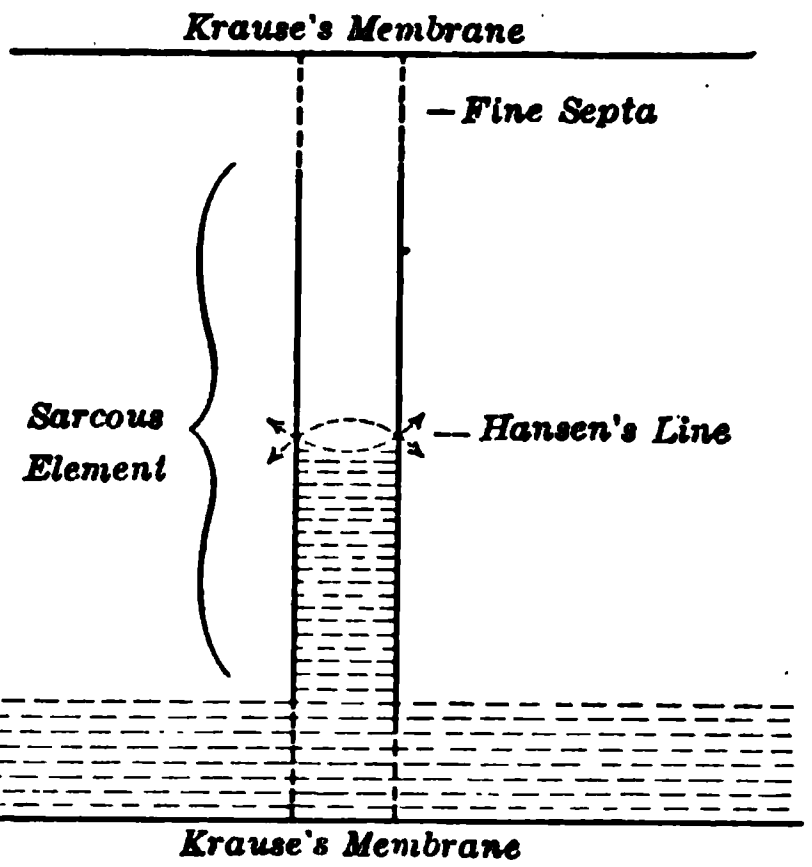

FIG. 26.—Schematic diagram of a muscle-element.

from the hyaloplasm will enter them. The sum of the effects of a multitude of such shortenings constitutes the contraction of a muscle-fibril.

The conception of a motile mechanism as a surface-tension engine may readily be extended to include ameboid and ciliary motion as well as the phenomenon of **Protoplasmic Streaming** which is so frequently displayed in cells in which ameboid motion is constrained by viscosity or by rigid walls, as in many plant-cells. The genesis of movements analogous on the one hand to ameboid motion and on the other to protoplasmic streaming may be illustrated in a simple model as follows: If to a ten per cent. solution of camphor in benzole a little dye, for example Sudan III or Scharlach R be added, to render the outline of a drop visible against a colorless background, and small drops of this be placed upon the surface of *clean* water in a watch-glass, very rapid and energetic movements of the edges of the drops may be observed exactly similar in character to those presented by the surface of *Ameba*.

Even processes similar in form to **Pseudopodia** are thrown out and retracted. These movements are due to the changes in interfacial tension caused by unequal diffusion of the camphor from the benzole into the water. They may be slowed by adding some viscous fluid, *e. g.*, olive oil to the benzol solution and finally, when about an equal volume of olive oil has been added, we no longer obtain ameboid movements but, instead, we observe an incessant streaming movement of the fluid within the drops, exactly resembling those seen within the protoplasm of a plant-cell such as *Chara*. If the streaming movements are not easily perceived owing to the transparency of the drop, the addition of a little finely powdered arrowroot starch will render them manifest, and impose a still more close resemblance to the actual appearance of streaming movements in protoplasm.

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CHAPTER XIX.

PROCESSES INFERRED FROM INDIRECT OBSERVATION: FERTILIZATION AND EARLY DEVELOPMENT.

THE SUBSTITUTION OF CHEMICAL AGENCIES FOR NORMAL FERTILIZATION.

In the vast majority of animal forms the stimulus of fertilization by a spermatozoön of the same or a very closely related species is essential for the development of the egg. The fact, however, that **Parthenogenesis**, or development without fertilization may occur under exceptional circumstances or in a limited number of forms, shows that the part played by the spermatozoön, in so far as it constitutes the stimulus to development, may be performed by other agents. The discovery of the exact nature of agents capable of giving rise to development of the egg was essential to the understanding of the phenomenon of **Fertilization**, for the spermatozoön, besides affording to the egg the initiatory impulse to development also acts as a bearer of hereditary factors and is, moreover, itself a living and a motile organism so that a great complexity of materials and factors gain entry into the egg with the introduction of the spermatozoön, and the disentanglement of these numerous variables was impossible until a clue to their nature had been obtained by means of experiments in which the single function of fertilization was imitated by physicochemical means.

The solution of this problem we owe to the investigations of J. Loeb who followed up the observation of T. H. Morgan and others that unfertilized eggs of various marine organisms may occasionally begin to segment without fertilization in sea-water, but that such eggs invariably die after a few divisions. In seeking to ascertain the origin of this abnormal phenomenon Loeb found that in the eggs of a sea-urchin, *Arbacia*, development could be induced by merely exposing them for a period to slightly **Hypertonic Sea-water** and then returning them to normal sea-water. The means employed to render the sea-water hypertonic was, within certain limits, immaterial. Thus the **Osmotic Pressure** might be raised by spontaneous evaporation, or by the addition of one part by volume of 2½ normal sodium chloride solution to nine parts by volume of sea-water, or yet again **Cane-sugar** or **Urea** or some other physiologically inert substance might be employed for this purpose and with like success. It was even found possible to cause development of the eggs by immersing them in a pure cane-sugar solution only slightly exceeding sea-water

in its osmotic pressure. The increase of osmotic pressure required is not great. If **Sodium Chloride** be employed an increase of forty per cent. in the osmotic pressure of the sea-water suffices to initiate development after an exposure of two hours. If sugar or urea be employed even a slighter increase of osmotic pressure suffices to bring about a like effect, because these substances penetrate the egg with greater difficulty than the inorganic salts and hence exert a greater osmotic tension on the external surface of the egg. The requisite concentration of the medium varies, however, with the duration of the exposure, a weaker concentration being effective after a longer exposure. This is shown by the following experiment: To 50 c.c. portions of artificial sea-water (**Van t'Hoff's Solution**) rendered favorably alkaline by the addition of 2 c.c. of tenth normal sodium hydroxide were added 0, 2, 4, 8 and 16 c.c. of 2½ normal potassium chloride solution. Unfertilized eggs of a Pacific Ocean sea-urchin (*Strongylocentrotus purpuratus*) were divided between these five solutions and samples removed after varying periods of exposure and placed in normal sea-water. The following were the results obtained:

Period of exposure, minutes.	Increase in the osmotic pressure of the medium.				
	0 per cent.	16 per cent.	30 per cent.	55 per cent.	87 per cent.
45	No larvæ	No larvæ	No larvæ	No larvæ	Numerous larvæ.
64	"	"	"	Numerous larvæ	
89	"	"	Numerous larvæ		
116 . . .	"	"			
114	"	"			

The fertilization which resulted from this procedure failed, however, to furnish a perfectly faithful imitation of the phenomenon of natural fertilization. It is true that the eggs frequently developed into free-swimming larvæ, but the larvæ produced in this manner were sickly and abnormal and did not survive very long. The percentage of eggs which developed into larvæ was variable and in some species, particularly in the sea-urchin *Strongylocentrotus franciscanus*, few if any of the eggs could be induced to develop by this procedure. The larvæ in all cases behaved abnormally; they did not rise to the top of the water and swim there as normal larvæ do, but swam instead at the bottom of the vessel containing them, and finally, the most marked peculiarity of all was the failure of the eggs to form a **Fertilization-membrane.**

If the eggs of a mature female sea-urchin be removed from the ovaries by shaking them out in sea-water and are then mixed with sperm similarly procured from the spermaries of a male, the spermatozoa will immediately be seen clustering around the eggs, presenting the appearance of striving to enter them. Within a very brief period, under normal conditions, a spermatozoön will succeed in effecting an entry, and this event is at once indicated by the appearance upon the

surface of the egg of a number of irregular clear blister-like protuberances which rapidly increase in number and extent, finally covering the surface of the egg with a clear hyaline layer, which is designated the fertilization-membrane (Fig. 27). This was lacking in the artificially fertilized egg.

The imperfect character of the imitation of fertilization which was thus achieved led Loeb to form the supposition that the osmotic method of inducing fertilization only accomplished a part of the effects initiated by the spermatozoön, which he inferred carried into the egg agencies not only capable of starting the processes initiated by the hypertonic sea-water but also processes which the osmotic method did not suffice to initiate. This supposition was confirmed by the discovery of a series of agents capable of inducing **Membrane-formation** in the sea-urchin egg.

1. 2.

FIG. 27.—1, unfertilized egg of the Sea-urchin (*Strongylocentrotus purpuratus*) surrounded by spermatozoa, 2, the same egg about two minutes later, after the entrance of the spermatozoön and the formation of the fertilization-membrane. (After Loeb.)

It was found that if mature sea-urchin eggs were introduced for a few minutes into sea-water to which a small proportion of a certain sample of **Ethyl Acetate** had been added, and then returned to normal sea-water, all of the eggs promptly formed a fertilization-membrane differing in no perceptible degree from the membranes formed in normal fertilization. Other esters failed to yield any comparable result, and an examination of the ethyl acetate employed in the original experiment showed that it had undergone hydrolysis and contained free ethyl alcohol and acetic acid. This led to an investigation of the behavior of the eggs in sea-water containing added alcohols and acids and to the discovery that the effect originally obtained with impure ethyl acetate was due to the **Acetic Acid** which it contained. It was found that all of the monobasic fatty acids which are soluble in sea-water, namely formic acid, acetic acid, propionic acid, butyric acid, valerianic acid and so forth, will induce membrane formation in 100 per cent. of mature eggs if they are exposed for a brief period to the action of the sea-water containing the fatty acid. The formation of the membrane

does not occur until after the restoration of the egg to the normal sea-water.

The eggs which have been treated in this manner may undergo a few divisions but they very rapidly die, more rapidly, in fact, than unfertilized eggs exposed to similar conditions of temperature, etc. The processes thus initiated therefore, still afford an incomplete analogy to natural fertilization. It was found, however, that by a combination of these two processes, membrane-formation and osmotic treatment, which are separately incomplete, a perfect imitation of fertilization is procured and a high percentage of the eggs, usually 100 per cent., can be induced to develop and produce normal larvæ.

The precise details of time, of exposure, concentration and so forth, in Loeb's improved method of **Artificial Parthenogenesis** necessarily vary slightly with the temperature, reaction of the sea-water and species of Echinoderm employed. The following are, however, the details of the method as utilized for the fertilization of the Pacific sea-urchin, *Strongylocentrotus purpuratus* at a temperature in the neighborhood of 15° C. The eggs, after extraction from the ovaries and rinsing in filtered[1] sea-water are immersed in a mixture of 50 c.c. of sea-water and 2.8 c.c. of tenth normal **Butyric Acid** solution, and the mixture is gently agitated to prevent the eggs, which become sticky, from adhering to the bottom of the vessel. After about two minutes the eggs are collected by gentle rotation of the shallow flat-bottomed vessel and transferred by means of a pipette to normal sea-water. If the exposure has been rightly chosen it will be found that the eggs almost immediately form membranes. After allowing them to remain for some fifteen to twenty minutes in the normal sea-water they are again collected in the manner described and transferred to **Hypertonic Sea-water**, prepared by adding 8 c.c. of 2½ molecular sodium chloride solution to 50 c.c. of sea-water. They are exposed to this addition for a period varying from fifteen to sixty minutes, the optimal exposure varying somewhat with the eggs from different females. The eggs are now returned to normal sea-water. Within about one hour the first cell-division will be observed to have occurred, at the end of forty-eight hours swimming gastrulæ will have been produced, and about two days later plutei with well-developed skeletons.

Artificial fertilization has been extended to a variety of forms other than the Echinoderms. In a number of *Annelids* development may be induced by preliminary treatment with a cytolytic agent followed by treatment with hypertonic sea-water. As a rule, however, in this group the fatty acids do not constitute sufficiently potent cytolytic agents, and saponins or, better still, mammalian blood serum must be employed. Among the *Molluscs* simple treatment with hypertonic sea-water frequently suffices, especially if it be rendered slightly hyper-

[1] The sea-water must be filtered to remove spermatozoa which may possibly be suspended in it.

alkaline, the alkali playing the part of the cytolytic or membrane-forming agent. In the eggs of frogs simple puncture with a fine needle suffices to induce parthenogenetic development, for what reason is not at present clearly understood, although we may fairly infer that it arises from the incidental admixture of certain constituents of the eggs which are normally separated from one another. Artificial parthenogenesis has also been induced in the eggs of plants (*Fucus*) by treating them with butyric acid, followed by hypertonic sea-water.

The eggs of all forms which have been made to undergo development by artificial means yield normal embryos and their development differs in no wise from that of normally fertilized animals. The rearing of marine animals is an excessively laborious task, but Delage has had the courage to undertake it in the case of artificially fertilized sea-urchins and succeeded in maintaining them until sexual maturity. In the case of the frog several specimens arising from artificially fertilized eggs have been brought to sexual maturity by Loeb and Bancroft (see Fig. 28).

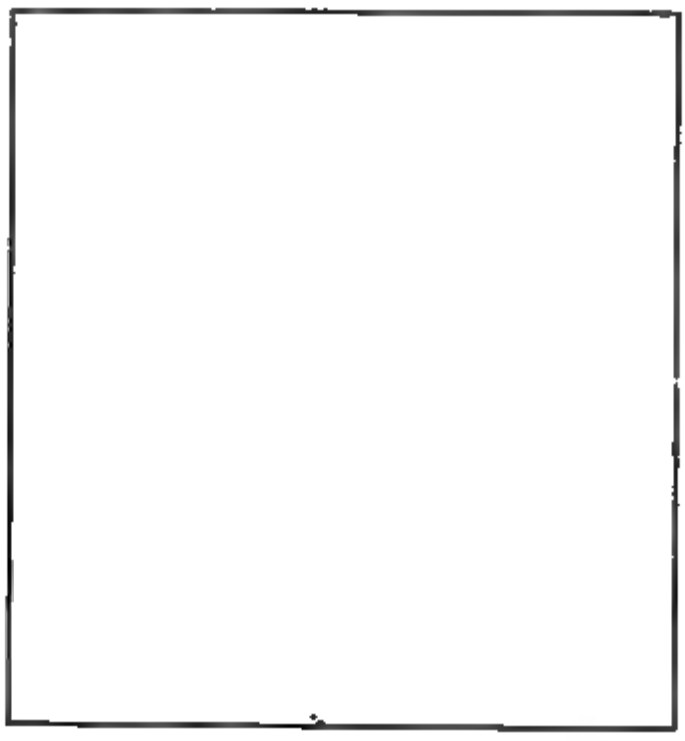

FIG. 28.—A parthenogenetic frog. (After Loeb.)

THE NATURE OF THE AGENTS WHICH FORM FERTILIZATION-MEMBRANES.

The monobasic acids of the fatty series are all capable of producing, as we have seen, the formation of membranes in the sea-urchin egg provided they are soluble in sea-water. Now this action might conceivably be due to the dissociation of **Hydrogen Ions** by the acids, or it might be due, on the other hand, to the anion or the undissociated molecule of the acid. The latter is the correct alternative, for although the highly dissociated mineral acids will induce **Membrane-formation** in a limited percentage of eggs, the requisite concentration of these

acids is far greater than it is in the case of the fatty acids. In fact one-thousandth normal **Butyric Acid** is more efficient in inducing membrane-formation than twelfth normal **Hydrochloric Acid**; yet hydrochloric acid of even this concentration does not injure the eggs in the periods of exposure requisite, because normal fertilization by sperm can still occur in 100 per cent. of the eggs treated in this manner, and weaker concentrations of hydrochloric acid, which are usually ineffective in causing membrane-formation, are, of course, even less toxic. A very convincing experiment devised by Loeb to illustrate this point consists in adding a little **Sodium Butyrate** to a solution of hydrochloric acid in sea-water which is otherwise incapable of causing membrane-formation. The mixture immediately becomes an effective membrane-forming agent, although its acidity, if anything, has been reduced. The introduction of the sodium butyrate leads to an interaction with the hydrochloric acid, setting free a little butyric acid which accomplishes the initial stage of fertilization.

It had been observed in 1887 by O. and R. Hertwig that if sea-urchin eggs be immersed in sea-water saturated with **Chloroform**—and only a trace of this substance will dissolve in sea-water—fertilization-membranes are formed. It had also been found by Herbst that **Benzene, Toluene** and **Creosote** have a similar action. In all these cases, however, the membrane-formation was found to be rapidly succeeded by **Cytolysis** and the disintegration of the eggs, so that development, of course, did not occur. Loeb found however, that if the eggs be exposed only for very brief periods to these solutions and then transferred to normal sea-water a percentage of the eggs will form membranes without cytolysis and may subsequently be induced to develop by treatment with hypertonic sea-water. It is a curious, and as yet unexplained fact that whereas eggs immersed in butyric-acid sea-water do not form fertilization-membranes until they are transferred to normal sea-water, eggs treated with sea-water containing benzene or amylene form membranes before they are removed from the mixture.

The various reagents which were thus found effective in inducing membrane-formation have this in common, that they are all fat-solvents or highly soluble in fats and furthermore they are all in greater or less degree **Hemolytic Agents**, that is, substances which are capable of dissolving red blood-corpuscles. This fact drew attention to the possibility that other hemolytic agents might be capable of exerting a like effect upon the unfertilized egg of the sea-urchin.

According to Koeppe, besides heat and alternating electric currents there are five distinct groups of chemical agents which are distinguished by their power of inducing hemolysis of red blood-corpuscles or, more generally, **Cytolysis** of all types of living cells. These are: 1. Certain specific substances, for example the series of glucosides comprising the **Saponins** and **Solanins**, or the **Bile-salts**. 2. A series of **Fat-solvents** such as benzene, ether or alcohol. 3. Distilled water. 4. Hydro-

gen ions and 5. Hydroxyl ions. Successive experiments have shown that each of these groups of reagents may, by appropriate manipula-

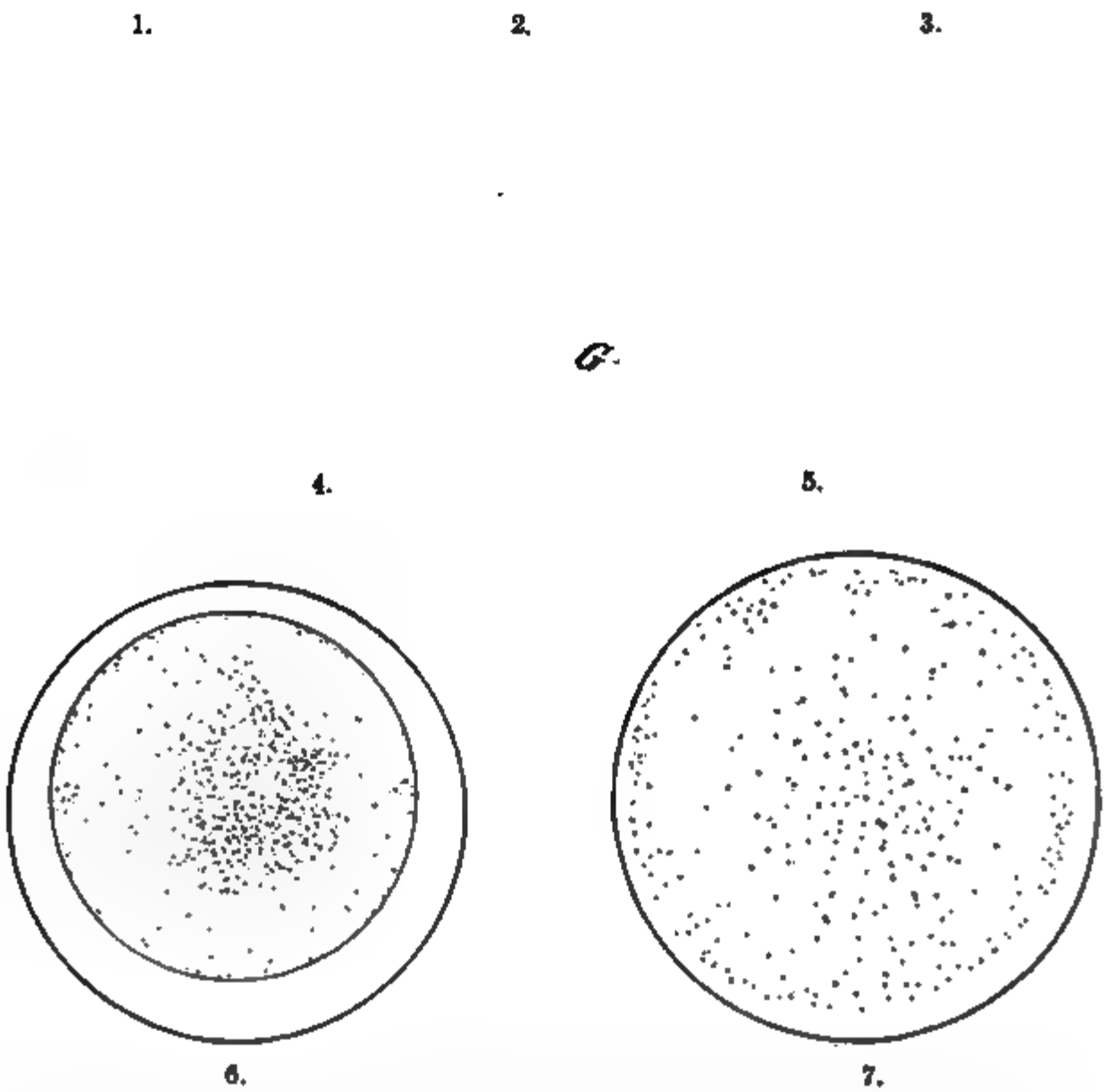

FIG. 29.—Formation of fertilization-membrane and cytolysis of the sea-urchin egg on treatment with saponin. 1, appearance of egg when first exposed to saponin solution at 9.07 A.M.; 2, 3, and 4, formation of fertilization-membrane. The stage of complete membrane-formation as depicted in 4 was reached at 9.15 A.M. (if at this stage the egg is withdrawn from the influence of the saponin it can develop). Cytolysis, 5, began at 9.20 A.M.; 6 and 7, advanced stages of cytolysis induced by saponin. (After Loeb.)

tion, be made to cause membrane-formation in the unfertilized sea-urchin egg. The **Saponins** especially are extraordinarily efficient in inducing this phenomenon, membranes being formed in 100 per cent.

of eggs by a dilution of one in a hundred thousand after an exposure of from 30 to 60 minutes; these membranes are formed in the solution itself without transference to normal sea-water. If, however, the action of the saponin solution upon the egg be permitted to continue, membrane-formation is rapidly succeeded by cytolysis and the egg disintegrates. This is illustrated in the preceding figure (Fig. 29), showing the successive effects of a saponin solution (8 drops of ¼ per cent. saponin to five c.c. of sea-water) upon the unfertilized eggs of *Strongylocentrotus*. It is perfectly clear from these results that the formation of the fertilization membrane represents an initial stage of cytolysis. If, however, the eggs be removed from the saponin solution before manifest cytolysis has occurred, washed in sea-water a number of times to remove the last traces of saponin, and then treated with hypertonic sea-water, normal development occurs and a large proportion of the eggs develop into larvæ. Precisely the same results are obtained with **Bile-salts.**

There remains, however, another class of cytolytic agent which has yet to be considered in this connection, namely the tissue-fluids of unrelated species of animals. The **Blood** of the mammalia contains cytolytic substances which hemolyze and destroy foreign corpuscles and cells, but not those of the same species. This cytolytic power of blood and other tissue-fluids is greatly enhanced by previous immunization with the foreign cells, but within the body at least it is exercised without previous immunization. It was found by Loeb that this class of cytolytic agents is also capable of causing membrane-formation in the sea-urchin egg. Not every unrelated species of animal, however, would furnish a tissue-fluid or extract capable of causing membrane-formation, in fact only a limited number of forms were found to do so. Loeb considers that this is due to the variable permeability of the eggs for different lysins, and the permeability of the eggs for a particular lysin also seems to vary somewhat in the eggs of different females.

The first cytolytic agent of this kind to be discovered was that contained in the blood of certain marine worms, namely *Dendrostoma*, which calls forth membrane-formation in the sea-urchin egg even if it is diluted a thousand times or more with sea-water. Later investigations have shown that a number of other invertebrates yield tissue-fluids or extracts which will cause membrane-formation. Most interesting results were, however, obtained by Loeb with the blood of mammalia or birds.

The blood-sera of mammals (oxen, sheep or pigs) which have been rendered isotonic with sea-water by the addition of sodium chloride will induce membrane-formation in sea-urchin eggs, but not invariably nor in all of the eggs derived from different females. Eggs from one and the same female will form membranes in some samples of blood and not in others; and again, one and the same sample of blood will form membranes in the eggs of some females but not in others. As in the case of the saponins the membranes are formed in the solution itself,

and removal to normal sea-water is not essential, but, unlike the membrane-formation by saponins it is not followed, in undiluted blood at least, by subsequent cytolysis. As we shall see, however, dilution of the blood by sea-water enables the cytolytic effect to appear, completing the analogy to the action of the saponins. As in all the instances previously considered, membrane-formation is succeeded by one or two cell-divisions and then by death and disintegration of the eggs, unless they are treated with hypertonic sea-water which enables them to develop and give rise to normal embryos.

Loeb sought for the origin of the differing action of the various sera by endeavoring to modify it, and he found that preliminary heating of the sera greatly enhanced their ability to induce fertilization. Preliminary treatment of the eggs, however, was found to be even more effective. This treatment or "sensitization" of the eggs consists in exposing them for a brief period to an isotonic solution of a chloride of an **Alkaline Earth**, calcium, strontium or barium. Of the three, however, strontium is much the most effective; the efficiency of barium might possibly exceed even that of strontium if it were not for the fact that barium is also exceedingly toxic for the eggs, while strontium is almost harmless. After exposure to the **Strontium Chloride** and subsequent transference to isotonic blood-serum, membranes are formed in nearly every case upon 100 per cent. of the eggs. It has been shown by A. R. Moore that this sensitization by strontium is due, not to any irreversible change induced in the egg itself by strontium, but more probably to the actual presence of the strontium within the egg, for if the eggs after exposure to the strontium chloride solution be washed free of the solution by two or three changes of sea-water, their sensitiveness to blood-sera is lost.

The membrane-forming substance in blood-serum was found by Loeb to be remarkably resistant to heat. Exposure of ox-serum to a temperature of 73° for half an hour, which leads to coagulation of the serum-proteins, does not destroy its fertilizing power. Heating the serum to 100° does, however, destroy the active substance. The blood of *Dendrostoma* still retains a proportion of its membrane-forming power, even after having been heated rapidly to boiling point; more prolonged boiling (2 to 3 minutes), however, destroys its activity. The thermostabile character of the active substance at once distinguishes it from the **Alexin** or bactericidal substance which is present in mammalian blood-sera, for this is inactivated by heating to 56°.

The active substance in mammalian sera is not extracted by shaking the serum with **Ether**. If several volumes of **Acetone** are added to the serum, the precipitate which results, after drying, powdering and resolution in sea-water, retains the power of inducing membrane-formation. The substance is therefore precipitated by acetone.

A very minute and intensely active fraction may be prepared from mammalian blood-sera by the following procedure. One hundred c.c. of ten per cent. **Barium Chloride** solution are added, with constant

stirring, to each liter of serum. This mixture is kept in a cool place for forty-eight hours, when the supernatant fluid can be decanted. The residue is washed several times with large volmes of 2 per cent. barium chloride solution to remove all traces of serum. The precipitate is now stirred up with tenth-normal hydrochloric acid, warmed to 45° C. using 50 c.c. for the precipitate from each liter of serum. After stirring for an hour or more the mixture is centrifuged and to the clear fluid thus obtained an equal volume of tenth-normal sulphuric acid is added. This solution is allowed to stand at 40° C. for twenty-four hours and then thoroughly centrifuged to remove barium sulphate. To the clear fluid are added four or five volumes of acetone and the mixture is cooled for eight hours or more, at the end of which time the white flocculent precipitate has settled. It can then be collected on a hardened filter, washed with ether and dried over sulphuric acid. This preparation, which has been designated **Oöcytin** has been carefully examined by G. W. Clark, who finds that successive samples differ in elementary composition, showing clearly that it is a mixture of two or more substances. It gives all the protein reactions but also yields on hydrolysis notable quantities of **Hypoxanthine** and a **Pentose**, but only a trace of phosphoric acid. These products correspond to those which would be yielded by the **Nucleosides** or glucosidal fractions derivable from the nucleic acids by partial hydrolysis. The presence of appreciable amounts of this glucoside in the preparation is of peculiar significance when the glucosidal structure of the **Saponins**, which are similarly potent in inducing membrane-formation, is borne in mind.

The membrane-forming power of oöcytin acting upon eggs sensitized by strontium chloride is very great, comparable in fact with that of the saponins. It will induce membrane-formation at a dilution of one in five hundred thousand. The sensitizing effect of strontium is clearly seen to lie in the fact that it precipitates the oöcytin and so concentrates it within or upon the surface of the egg. In concentrated solutions eggs which have been freshly transferred from strontium chloride solution collect a dense precipitate at their periphery which may often mechanically prevent or delay the formation of the fertilization-membrane. That the activity of the preparation is not due to contamination by barium or other inorganic substances is shown by the fact that it is inactivated by heating for a few minutes to 80° C_t; the temperature at which the activity of blood-serum itself is destroyed.

From the spermatozoa of the sea-urchin, cytolyzed by distilled water, a similar fraction may be prepared having analogous potency in inducing membrane-formation in eggs which have been sensitized by immersion in strontium chloride solution. This material has also been found by Clark to yield notable amounts of a purine base and a pentose on hydrolysis. It would appear very probable therefore that membrane-formation in natural fertilization is brought about by the introduction into the egg, within the body of the spermatozoön, of a glucosidal cytolytic agent, which is related to the nucleosides.

The action of oöcytin upon the sea-urchin egg differs from that of isotonic mammalian blood serum in two respects; firstly in the fact that prolonged exposure of the eggs to oöcytin solutions causes cytolysis and secondly in the fact that it is potent at a great number of different dilutions whereas the potency of isotonic serum to induce membrane-formation disappears upon dilution of the serum to one-half, or at all events one-fourth, by the addition of sea-water. This difference in behavior is apparent, however, and not real. It is due to the inhibiting action of the proteins which are also present in the serum.

If isotonic blood-serum be diluted by successive additions of sea-water the membrane-forming power is at first weakened and then disappears, but upon further dilution it reappears and then is retained to very high dilutions. The following are illustrative experiments:

Ox-serum Sample III.

Eggs sensitized by four minutes' immersion in $\frac{3}{8}$ m. $SrCl_2$

Dilution of the isotonic serum.	Per cent. of membrane formed in fifteen minutes.	Per cent. of membrane formed in fifty minutes.
1	100	100
1/2	5	50
1/4	0	0
1/8	Not observed	44
1/16	"	100

Ox-serum Sample V.

Eggs sensitized by four minutes' immersion in $\frac{3}{8}$ m. $SrCl_2$.

Dilution of the isotonic serum.	Per cent. of membrane formed in fifteen minutes.	Per cent. of membrane formed in ninety minutes.
1	68	100 (none cytolyzed)
1/2	80	86 (none cytolyzed)
1/4	0	0 (none cytolyzed)
1/8	0	16 (1 per cent. cytolyzed)
1/16	1	72 (10 per cent. cytolyzed)

It is evident that when the membrane-forming power is regained in the higher dilutions the power of inducing cytolysis is also acquired, so that the action of the blood-serum now resembles that of saponin or oöcytin in every respect. The failure of cytolysis to appear in undiluted serum is due to the inhibiting effect of the high concentration of **Protein** which it contains, and even membrane-formation may be inhibited if the concentration of serum-proteins is too high or if additional protein be dissolved in the serum. Such sera will nevertheless induce membrane-formation and cytolysis if they are diluted, the inhibiting effect of the proteins becoming negligible at a dilution of one in sixteen or one in thirty-two.

Even membrane-formation by **Butyric Acid** or by spermatozoa may

be inhibited by the addition of proteins to the sea-water. The following table shows the relative efficiency of various proteins in inhibiting membrane-formation:

Protein.	Highest observed concentration which permits membrane-formation by butyric acid.	Lowest observed concentration which prevents membrane-formation by butyric acid.
Mixed serum proteins	3.7	7.4
Gelatin	1.0	2.0
"Insoluble" serum-globulin	0.3	0.6
Casein	0.25	0.5
Ovomucoid	0.125	0.25

It is a very striking fact that the order of effectiveness of these proteins in preventing the formation of membranes is the reverse order of their ability to pass through a porcelain filter. It has been suggested by Loeb and von Knaffle-Lenz that the formation of the fertilization-membrane is accompanied by the entry of water into the egg. This is prevented or delayed by the presence of colloids in the surrounding medium because they cannot penetrate the egg and hence exert an osmotic pressure tending to withdraw water from the egg. For similar reasons cytolysis is also inhibited and it has also been stated by B. Moore that the action of **Hemolytic Agents** in liquefying blood-corpuscles is similarly inhibited by proteins.

The normal concentration of protein in blood-serum lies between 7 and 8 per cent. and it will be seen that this lies in the margin of the concentration which inhibits membrane-formation by butyric acid (and also by sperm). Hence if the oöcytin content of a sample of serum be low, or the concentration of serum-proteins a little above the average, it will fail to cause membrane-formation even in sensitized eggs. Heating the serum permits membrane-formation to occur because it results in coagulating and removing the proteins, and dilution achieves the same result in a different way. At first, however, the effect of dilution in reducing the membrane-forming power of the serum more than compensates for the diminished inhibition by the proteins, so that dilution of serum to one-half or one-fourth usually deprives even an initially active serum of the power to induce membrane-formation. Even when the undiluted serum is sufficiently potent to overcome the inhibition of its proteins so far as to cause membrane-formation, the inhibition is nevertheless operative and finds expression in the prevention of the subsequent cytolysis.

The concentration of protein in the medium bathing the eggs which is required to inhibit membrane-formation affords a quantitative measure of the potency of the fertilizing agent. The more concentrated a solution of **Saponin**, for example, the greater the amount of **Ovomucoid** which must be added to it to prevent the formation of membranes. From this it is evident that the "charge" of membrane-forming agent which the spermatozoön carries into the egg must be less than that which is deposited upon sensitized eggs in an active serum

which induces membrane-formation without previous dilution, for the concentration of proteins in normal undiluted serum is sufficient to inhibit the membrane-formation succeeding fertilization by spermatozoa. It is, however, possible to increase the "charge" of membrane-forming agent in spermatozoa by sensitizing them with strontium chloride solution and exposing them to blood-serum previous to fertilization. They thus accumulate the membrane-forming agent from the serum and carry it together with their own membrane-forming agent into the egg. The following experiment affords an illustration of this fact. Solutions of ovomucoid in sea-water were prepared containing 2, ½, ¼ and ⅛ per cent. of the protein, respectively and in 2 c.c. samples of each of these solutions were placed two drops of a thick suspension of the eggs of *Strongylocentrotus purpuratus*. The sperm from a male of the same species was divided into three portions. The one portion was untreated save by washing in sea-water. A second portion was immersed for four minutes in ⅜ m. strontium chloride and then for one minute in sea-water. The third portion was immersed for four minutes in ⅜ m. strontium chloride and then for four minutes in an isotonic undiluted blood serum. These three samples of sperm were then added to the eggs contained in the solutions of ovomucoid in sea-water described above. The concentrations of ovomucoid employed inhibited the formation of fertilization-membranes by the normal spermatozoa and by those which had merely been immersed in strontium chloride solution, but, except in the case of the strongest solution, they were unable to prevent the formation of membranes by the spermatozoa which had acquired an additional charge of cytolytic substance from blood serum:

Concentration of ovomucoid solution.	Per cent. membranes formed by untreated sperm after:		Per cent. membranes formed by sperm washed in $SrCl_2$ and then in sea-water after:		Per cent. membranes formed by sperm washed in $SrCl_2$ and then in serum after:	
	15 mins.	40 mins.	15 mins.	40 mins.	15 mins.	40 mins.
2 per cent. . .	0	0	0	0	0	0
1/2	0	0	0	0	20	30
1/4	0	0	0	0	30	40
1/8	5	7	8	8	18	18

It appears, therefore, that the cytolytic agent in mammalian blood-serum when introduced into the egg-cell together with the spermatozoön, brings about just the same effects as the cytolytic agent in the spermatozoön itself and the inference is thus rendered the more probable that these two agents are similar in character.

THE EFFECT OF MEMBRANE-FORMING AGENTS UPON THE EGG.

The essential feature of the process of **Membrane-formation** is its evidently close relationship to the phenomena of **Cytolysis**, or liquefaction and disintegration of the cell. In the view of Loeb it is cytolysis

which is confined to the cortical layer of the egg. The formation of the membrane is accompanied by a very manifest increase in the volume of the egg which can only be accounted for by an imbibition of water. The cytolysis which succeeds membrane-formation is accompanied by a still greater swelling and imbibition of water. This has been attributed by Loeb and von Knaffle-Lenz to the partial liquefaction or destruction of an **Emulsion-structure** within the cell or at its periphery. We have seen (Chapter XIII) that protoplasm consists of an emulsion of lipoids in a protein medium and that this emulsion must be particularly concentrated at the surface of the protoplasm owing to the lowering of interfacial tension which is thus brought about. Any increase in the diameter of the lipoidal droplets in this superficial layer, or their coalescence, must lead to a corresponding increase in the width of the interstices between them, and hence to an enhanced permeability for water and salts. The fat-dissolving character of the majority of the cytolytic agents is thus the origin of the taking up of water by the egg which results in the physical phenomena of cytolysis or cell-liquefaction. Any other agent which will induce imbibition of water will, however, also bring about cytolysis. For example distilled water or **Hypotonic Solutions** bring about cytolysis because the excess of osmotic pressure within the egg forces water into the cell even through the normally narrow interstices of the cortical layer. Other agents may induce cytolysis by altering the solubility of the protein component of the emulsion, and hence cytolysis may be induced in certain cells by physiologically unbalanced salt solutions. Even membrane formation, as Lillie has observed, may be brought about in certain echinoderm eggs (*Arbacia*) by exposing them to pure solutions of certain **Sodium Salts**, and this effect is inhibited by an admixture of **Calcium Chloride**.

The formation of the fertilization-membrane, therefore, which is the first step in the stimulation of development which constitutes fertilization, is essentially a partial and arrested cytolysis. The important question now presents itself, in what way does this partial cytolysis affect the chemical processes of the cell?

One very decided effect of fertilization by spermatozoa is enhancement of **Basal Metabolism**, indicated by a greatly increased consumption of oxygen. The following measurements by Loeb and Wasteneys illustrate this fact:

CONSUMPTION OF OXYGEN BY A GIVEN MASS OF ARBACIA EGGS.

Time.	Mg. of oxygen consumed per hour.
Before fertilisation	0.24
First hour after fertilisation	0.94[1]
Second hour after fertilisation	0.80
Third hour after fertilisation	0.87
Fourth hour after fertilisation	0.91
Fifth hour after fertilization	1.05

[1] This value is too high, owing to the presence of sperm which were washed away before the next determination was made.

During the period occupied by the experiment the eggs had proceeded to the thirty-two cell stage. The rate of oxidations does not reach its maximum instantaneously, but increases progressively. For example, Warburg in comparing the rates of oxidation in the 8-cell and 32-cell stages found that they were in the ratio of 4.2 to 6.8.

Corresponding with these facts we find that deprivation of oxygen arrests the processes of development and prevents nuclear and cell-division. The same effect is brought about by **Cyanides**, which also arrest cellular oxidations and, in multicellular animals, act primarily by reducing tissue-respiration. It is possible to show, however, that other processes besides oxidations are initiated by fertilization, for when the fertilized eggs of *Strongylocentrotus purpuratus* are left in sea-water free from oxygen for twenty-four hours at 15° C. they will not develop during that time, but they will begin to develop at once if oxygen is admitted. It is found, however, that their development is no longer normal, since they form abnormal blastulæ and never or rarely reach the gastrula stage (Loeb). If unfertilized eggs are kept for twenty-four hours without oxygen they remain uninjured, and upon the addition of sperm they develop normally and produce healthy plutei. The same result is obtained if development is arrested by potassium cyanide.

It has been shown by Loeb in fact that not only is development arrested by deprivation of oxygen, but it is also, to some extent, *reversed*. Thus if development be initiated by membrane-formation with butyric acid in *Arbacia* eggs, on restoring the eggs to normal sea-water they die within a few hours unless they are treated with hypertonic sea-water; moreover, they are no longer fertilizable by sperm. However, if, instead of transferring the eggs to normal sea-water, they are placed in sea-water containing sodium or potassium cyanide, or chloral hydrate, then after some hours they no longer die when they are returned to normal sea-water and, in fact, may now be fertilized by sperm.

The fact that **Chloral Hydrate** and other narcotics, as well as cyanides, will arrest the development of fertilized eggs is a striking proof, in itself, that other chemical phenomena besides oxidation underlie development, for the narcotics, although they suppress or retard the processes of cell division and development do not perceptibly diminish the rate of oxidation in the egg. The acceleration of basal metabolism which occurs in fertilization, therefore, although essential to development, is not the only essential chemical transformation which underlies the process of development. The vast majority of the reactions which occur in living tissues are oxidations, reductions, or hydrolyses[1] and we may therefore consider it probable that **Hydrolysis** also occurs and performs an essential function in early development.

It remains now to discuss the relative parts played by the two factors of fertilization, the one consisting in the partial cytolysis of the

[1] Decarboxylization should perhaps be added to this list. Deaminization may with propriety be classed among the hydrolyses.

egg and the other, which is also brought about by the spermatozoön and may be imitated by treating the eggs with hypertonic sea-water. In the first place, as regards the cytolytic effect, or **Membrane-formation** it has been found that the characteristic acceleration of **Oxidations** which is induced by complete fertilization is also induced by membrane-formation. Thus Warburg compared the rate of oxidations in unfertilized eggs and in eggs which had been fertilized by sperm, and he found that the consumption of oxygen in the eggs which had been fertilized was 10.5 times the consumption of oxygen in the unfertilized eggs. The same eggs after butyric acid treatment consumed 9.0 times as much oxygen as the unfertilized eggs; the effect of membrane-formation alone upon the basal metabolism was therefore very nearly equal to that of complete fertilization. These experiments were repeated by Loeb and Wasteneys who, in another species of sea-urchin, found the ratio of oxygen-consumption in unfertilized and sperm-fertilized eggs to be 1:4.55, while two estimations of oxygen-consumption in the same unfertilized eggs after membrane-formation by butyric acid gave the values 1:4.72 and 1:4.28, indicating that the effect of membrane-formation is to raise the rate of oxidations to approximately the same height as the entrance of a spermatozoön.

We have seen that membrane-formation is essentially a partial and arrested **Cytolysis**. That this is really the essential feature in the process and not merely an incidental phenomenon is shown by the fact that if cytolysis be pushed even further, by whatever agent it may be caused, the effect is to increase the consumption of oxygen by the egg and approximately in proportion to the degree of cytolysis which is induced. Complete cytolysis of the egg of the sea-urchin can be caused by the addition of **Saponin** to the sea-water. Loeb and Wasteneys measured the rate of oxidations in a batch of unfertilized eggs in sea-water and they found that they consumed 0.15 mg. of oxygen per hour at 15° C. The eggs were then cytolyzed with saponin and the amount of oxygen consumed per hour at 15° C. determined again. It was found to be 1.07 mg. The complete cytolysis of the eggs, therefore, increased the rate of oxidation 700 per cent., or rather more than fertilization itself. Cytolysis by hypotonic sea-water also causes an increase in oxidations.

The second factor in artificial fertilization, the treatment with **Hypertonic Sea-water**, also increases the consumption of oxygen by the egg, but only to a relatively slight degree, and not at all if it succeeds membrane-formation whether induced by a spermatozoön or by butyric acid. Thus Loeb and Wasteneys obtained the following results with the unfertilized and otherwise untreated eggs of *Strongylocentrotus purpuratus:*

Solution.	Oxygen-consumption in ninety minutes, mgm.
Normal sea-water	0.30
Hypertonic sea-water (= 50 c.c. sea-water + 9 c.c. 2½ m. NaCl + KCl + $CaCl_2$)	0.67
Normal sea-water half an hour later	0.51
Normal sea-water twenty-one hours later	0.48

The increase in oxygen-consumption is obviously much less than that caused by membrane-formation and it is, moreover, transitory, falling off with time after the exposure instead of increasing, as it does when the eggs are normally fertilized or treated with butyric acid. On the other hand in eggs in which membrane-formation has been induced by butyric acid or by the entry of a spermatozoön, no increase whatever and no important decrease in the rate of oxidations could be observed on treatment with hypertonic sea-water. The corrective effect of hypertonic solutions in preventing the death and disintegration of the eggs which succeeds membrane-formation by cytolytic agents is therefore not to be sought in an effect upon oxidations. It may possibly reside in an effect upon underlying hydrolyses, for these, as we have seen, will bring about the destruction of the egg if they are permitted to go forward while the oxidations are retarded or prevented by lack of oxygen or by cyanides, and hence if they were disproportionately rapid, even in eggs in which oxidation were proceeding they might be presumed to exert a like deleterious effect. However this may be, the action of the hypertonic solutions upon the egg is not reversible upon restoration to normal sea-water. The effect is to induce a permanent alteration of the egg which renders it able to withstand partial cytolysis (membrane-formation) without injury. Thus Loeb has shown that if the unfertilized eggs of *Strongylocentrotus purpuratus* be placed for from two to two and a half hours in hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2½ m. Ringer solution) they may be returned to normal sea-water and subsequent treatment with butyric acid, even forty-eight hours later, will induce, not merely membrane-formation, but full and normal development of the embryo.

THE RELATIONSHIP OF PHOSPHOLIPINS TO THE SYNTHESIS OF NUCLEAR MATERIAL AND THE EFFECTS OF LECITHIN UPON EARLY DEVELOPMENT.

The leading results of the early development of the embryo are, in the first place, the very great increase of cellular surface due to repeated subdivisions of the original egg-cell and in the second place an increase in the proportion of nuclear to cytoplasmic constituents. The earlier estimations of Boveri led him to the conclusion that the mass of nuclear material in the cells is doubled at each cell-division, but the more recent estimations of Conklin have tended to greatly reduce this estimate, the average nuclear growth during cleavage amounting, it appears, to not more than from five to nine per cent. for each cleavage that occurs. Nevertheless there is a definite increase in nuclear material during the formation of the multitude of new cells which comprises the **Blastula-stage** of the sea-urchin and since during this period of development no growth of cytoplasm occurs, the cytoplasm of the new cells occupying collectively the same space as the original egg-cell, it is evident that a disproportion of nuclear to cyto-

plasmic material must be established, a disproportion which subsequent development corrects.

The **Synthesis of Nuclear Material** is a self-accelerated or autocatalyzed phenomenon. This follows from the fact that each successive cell division occupies about the same length of time as the preceding one, but the number of nuclei which results from the divisions is at each division twice as great as in the preceding one. The rate of production of nuclei therefore forms a geometrical progression in time intervals which constitute an arithmetical progression. The synthesis of nuclear material thus evidently accelerates the formation of fresh nuclear material. (Loeb.)

The question now presents itself as to the origin of the materials from which the nuclei are synthesized. The most characteristic constituent of the nucleus is **Nucleic Acid** which is built up by the combination of purine bases, a carbohydrate radical and phosphoric acid. The derivation of the first two of these components from proteins and from carbohydrates previously present in the egg is readily conceivable but the question of the origin of the phosphoric acid component suggests several interesting possibilities. In the first place it is evidently not derived from the external medium which bathes the cells, for perfectly normal development will occur in **Van t'Hoff's Solution**, which contains no phosphates. The phosphoric acid which is required for the synthesis of nucleins must therefore be derived from some constituent of the egg-cell. Two groups of constituents present themselves as abundant sources of phosphoric acid, namely inorganic phosphate and the **Phospholipins**, of which egg-lecithin may be taken as a type. Now we have no evidence whatever that nucleic acid can be synthesized directly from inorganic phosphates, but we have, on the contrary, a great deal of evidence which goes to show that phospholipins contribute in the synthesis of nuclear materials. Thus, Miescher has shown that during spermatogenesis in the salmon the "lecithin"-content of the tissues diminishes, Hoppe-Seyler has pointed out that the Lecithin-content of embryonic tissues is exceptionally high and Mesernitzky and Plimmer and Scott and others have shown that the lecithin-content of hen's eggs which is initially very high, progressively diminishes during the development of the embryo. That the same process occurs in the development of the sea-urchin egg has been shown by Robertson and Wasteneys, who estimated the proportion of alcohol-soluble phosphorus in eggs which had just been fertilized and again in eggs which had developed to blastulæ and plutei. The following were the results obtained with the developing eggs of *Strongylocentrotus purpuratus:*

	Percentage of the total phosphorus present in alcohol-soluble forms.	
Stage of development.	Experiment I.	Experiment II.
Fertilized eggs	39.5	46.5
Blastulæ	36.5	38.8
Plutei	35.2	35.1

In the first experiment the alcohol-soluble phosphorus (phospholipins) decreased by one-eighth, in the second by one-fourth, and this decrease was progressive. The experimental evidence from a diversity of forms therefore tends to establish a relationship between the disappearance of lecithin or other phospholipins and the synthesis of nuclear materials.

This being the case, great importance attaches to the fact that lecithin, when added to the medium in which sea-urchin embryos are developing, strongly retards their development. The fertilization-membrane is dissolved by lecithin,[1] and hence if lecithin in sufficient concentration (0.15 per cent.) be added to sea-water containing recently fertilized eggs, the membranes are disintegrated and the cleavage-cells which have been formed fall apart, so that for merely mechanical reasons further development is an impossibility. If more dilute lecithin solutions are employed this does not occur, but, at the same time, no effect upon the rate of development is observed. Very different results follow the exposure of the developing eggs to lecithin solutions, however, after the fertilization-membrane has in the normal course of development undergone rupture and liberated free-swimming blastulæ. The following experiment is illustrative of the phenomena which are then observed: The eggs of a *Strongylocentrotus purpuratus* female were divided into two portions. Both portions were placed in sea-water and fertilized with sperm. After twenty-four hours both lots of eggs had developed into free-swimming blastulæ. One portion was now transferred to a mixture of fifty c.c. of sea-water and 5 c.c. of a 1.7 per cent. emulsion of egg-lecithin in $\frac{m}{5}$ sodium chloride solution for a period of twenty-four hours and then returned to normal sea-water. The other portion was left in normal sea-water. The following table shows the relative development of the two portions:

Time after fertilisation. Days.	Portion 1 (controls).	Portion 2.
1	Blastulæ	Blastulæ (these were now transferred to the lecithin mixture for twenty-four hours).
2	Gastrulæ	Blastulæ (these were now transferred to normal sea-water).
3	Gastrulæ	Blastulæ.
4	Gastrulæ and early plutei	Blastulæ.
5	Fully developed plutei	Blastulæ and 25 per cent. gastrulæ.
6	Advanced plutei	Early gastrulæ with narrow unbranched intestine and large clear body-cavity.
7	Advanced plutei	Unchanged.
8	Advanced plutei	The gastrulæ are now retrograding; the intestine has almost disappeared.
9	Unchanged	Unchanged.

[1] We may infer from this that the periphery of the fertilisation-membrane contains lipoidal constituents which are essential to the integrity of its structure.

It is evident that the immersion of the blastulæ for twenty-four hours in a 0.15 per cent. solution of lecithin enormously retards their development. Especially remarkable is the fact that after development has actually proceeded to the gastrula stage it shows a tendency to undergo reversion, retracing the course of development to the blastula stage.

If *purpuratus* eggs are fertilized by sperm in more dilute solutions of lecithin in sea-water (0.003 per cent. to 0.015 per cent.) the fertilization-membranes are not dissolved sufficiently rapidly to affect development. In these solutions, as has been stated, development is not appreciably retarded until the blastula stage is reached, probably for the reason that colloids cannot traverse the fertilization membrane, and hence the lecithin cannot penetrate the cells of the embryo until the fertilization membrane has been ruptured. Thereafter development is very markedly retarded and the retardation is greater the greater the concentration of the lecithin. The eggs are not injured by the lecithin, however, as they will ultimately develop to normal plutei if left in these solutions for a sufficient length of time.

The action of **Cholesterol** is so very generally antagonistic to that of lecithin that one might anticipate that it would, as in fact it does, antagonize the effects of lecithin upon the development of sea-urchin eggs. If cholesterol, suspended in a mixture of $\frac{m}{100}$ sodium oleate and $\frac{m}{2}$ sodium chloride be mixed with lecithin in equal proportions the retarding action of the lecithin upon the development of sea-urchin eggs is almost completely neutralized. The slight retardation which is observed in these mixtures may be due to the **Sodium Oleate** which is employed to keep the cholesterol in suspension, since sodium oleate is very toxic for sea-urchin eggs and embryos.

Cholesterol itself, when added to sea-water, has no influence upon the rate of development of the eggs. The emulsions of cholesterol are, however, coagulated by the salts in sea-water and the cholesterol is completely thrown out of suspension in the form of coarse flocculi.

Since the preparations of "lecithin" employed in these experiments simply consisted of the mixture of phospholipins which is thrown out of an ether extract of egg-yolks by the addition of acetone, it cannot be definitely decided whether the effects observed were in reality due to lecithin or possibly to some other **Phospholipin** present as an admixture in these preparations. The significant feature of these results lies, however, in the fact that if the phospholipins within the egg itself and in other developing tissues behave similarly to the "lecithin" from yolks of eggs, then their progressive disappearance during nuclear synthesis must result in a proportionate diminution of their retarding effect, so that the auto-acceleration of nuclear synthesis, alluded to above, may wholly or in part be due to the consumption of phospholipins which is incidental to the process; progressive removal of a retarder being, of course, equivalent in its effect to the progressive addition of a catalyzer.

THE CHEMICAL MECHANICS OF CELL-DIVISION.

The essential mechanical resultant of cell-division is the increase of the **Protoplasmic Surfaces** which is brought about. During the successive divisions which an egg-cell undergoes in developing to the blastula-stage the total area of the protoplasmic surfaces is enormously increased even in attaining the thirty-two cell stage, for example, the total protoplasmic surface is increased by about three hundred per cent. Such an increase in a fluid surface necessarily implies either the performance of work by external forces or else a considerable *reduction* of **Superficial Tension.**

In 1876 it was suggested by Bütschli that cell-division is brought about through an *increase* of surface-tension, subsequent to nuclear division, in the equatorial region of the egg. He pointed out that substances diffusing from the nuclei or centrosomes must necessarily reach their highest concentration in the equatorial plane and hence assuming that these substances increase the surface-tension at the periphery of the egg, the most marked increase would occur in the equatorial region and, as a consequence, the surface-tension at the poles of the cell would be less than that at the equator.

Such a conception of cell-division is, however, manifestly erroneous for an increase of interfacial tension at the equator, such as Bütschli imagines to occur, implies an increase in the molecular attractive forces at the equator, and the fluids of the cell would not stream away from the region of high attraction but would, on the contrary, stream toward it. The result would be that the equatorial surface would tend to become highly curved, as areas of high tension in a fluid always do, and the surfaces at the poles would become relatively flattened. The result would be the formation of a flattened disc with a highly curved edge, the latter representing what was formerly the equatorial surface of the egg. Such a process obviously could not lead to cell-division. In fact, since the surfaces which Bütschli imagines streaming from the nuclei or centrosomes are supposed by him to raise the surface-tension of the egg, their total effect could only be to diminish the surface of the egg relatively to its volume, if that were possible, and not to increase it, which is what the forces leading to cell-division actually accomplish. In fact no model can be imagined in a fluid which will accomplish increase of surface by increase of superficial tension. Such a model can be devised in a non-fluid system, as, for example a rubber balloon subjected to compression by a rubber band around its equatorial circumference. The equator of the balloon would by this means be constricted and the single sphere would tend to divide into two, owing to the application of additional tension at its equator. This model is, however, in no way comparable to a fluid drop, for it is characteristic of the superficial tension of liquids that it is not altered by diminution or expansion of the surface, because it is really due to the unbalanced attraction of the underlying molecules of the liquid for each other. If a cleft is formed in a liquid drop the opposite walls of the cleft attract

one another and tend to close up the cleft again. If they fail to do so it can only be because the molecular attractions have been weakened, *i. e.*, the surface-tension diminished. In a rubber balloon there is no such attraction across the cleft; the tension is purely transverse and is not exerted perpendicularly to the surface as it is in a fluid, and so there is nothing to prevent a cleft from extending deeper and deeper into the equator of the rubber balloon provided the tension of the encircling band is reduced thereby to a greater extent than the tension of the balloon is increased.

In 1895 it was suggested by Loeb that phenomena of **Protoplasmic Streaming** are what really lead to cell-division. He pointed out that in cell-division the protoplasm streams from the equator of the cell in opposite directions toward the two nuclei; the violence of these streaming movements, he suggested, brings about the mechanical separation of the two cells.

The streaming of protoplasm from the equator toward the poles suggests that the phenomenon antecedent to cell-division is a diminution of surface-tension in the equatorial region and not, as Bütschli suggests an increase. That such equatorial diminution in surface-tension will bring about the division of droplets into two is very readily shown by means of the following simple experiment:

The formation of **Soaps** at the surface of oil-droplets, results as we have seen in Chapter XIII, in a diminution of the surface-tension of the droplets; if the formation of soap is local, that part of the surface upon which the soap is formed tends to spread. Since commercial olive oil almost invariably contains traces of **Fatty Acid** the result of bringing an alkali in contact with a drop of such oil will be the formation of soap at the points of contact. If, now, a drop of **Olive Oil** which is not too large (about 2 to 3 mm. in diameter) be floated on a layer of water, and a thread saturated with tenth normal alkali (NaOH or KOH) be brought gently into contact with a diameter of the drop, the almost immediate effect is the division of the drop into two. The phenomena accompanying this division are perfectly characteristic. Instantly the edges of the drop (the ends of the diameter along which the thread lies) recede from the thread, forming a notch at each end of the diameter, and violent streaming-motions occur at the surface away from the thread and toward the opposite poles of the drop. These streaming movements may be so violent as to rotate the droplets into which the drop divides through as much as 360°. If the division does not occur too rapidly the streaming may result in the two droplets being connected by a thread of oil, which may be central or to one side, and it may then be clearly seen that the mechanism which brings about the snapping of this thread is the violent streaming in opposite directions which takes place in the drops. Phenomena almost exactly resembling those described by Loeb in dividing ova may readily be observed (Fig. 30). Frequently, also, processes resembling **Pseudopodia** are thrown out by the droplets in the act of their division.

The segmentation of the drop is not due to mechanical division by

the thread, for, in the first place, the streaming phenomena, etc., are obviously attributable to soap-formation, and, in the second place, the phenomena observed when a thread, unwetted save with water, is laid across the drop are quite different from those described above. The drop of oil adheres to the thread and forms an elongated ellipsoid, its long axis coinciding with the thread; in fact the drop of oil assumes somewhat the form which the cell would assume were the phenomenon subsequent to nuclear division, as Bütschli imagines, an *increase* in surface-tension at the circumference of the equatorial layer.

Similar phenomena may be obtained with submerged droplets, formed by adding chloroform to the oil to increase its specific gravity, or by droplets immersed in a column of salt solution of varying concentration the lower layers being saturated, so that the drop floats midway without sinking or rising, only in this case stronger alkali must be used because the greater part of it is washed off the thread in passing it down to the drop through the upper layers of water or salt solution.

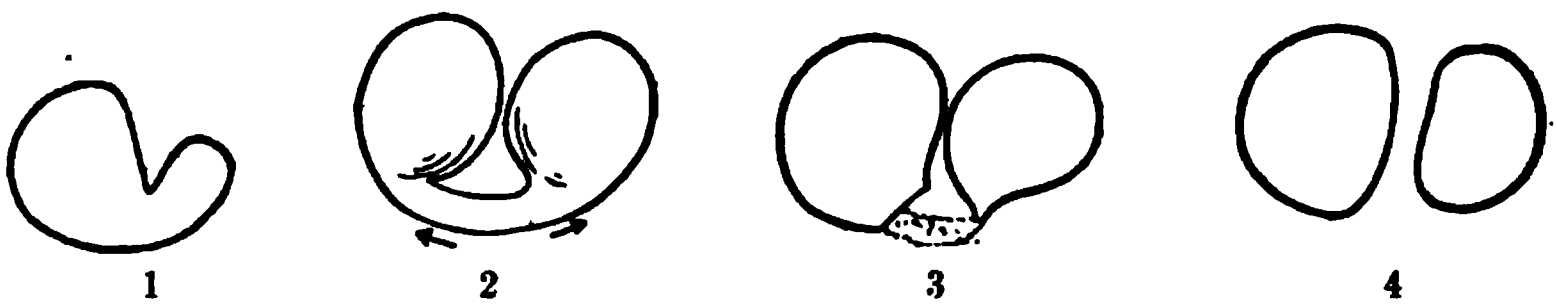

FIG. 30.—Drawings of a case of cell-division in artificial parthenogenesis (sea-urchin egg) illustrating the underlying phenomenon of streaming. "The division began on one side (1) and the protoplasm then flowed in the direction of the arrows (2) in opposite directions toward the two nuclei. The connecting-piece becomes empty of protoplasm and only the pigmented solid surface film is left (3) and finally this also disappears (4)." (After Loeb.)

The action of alkalies is not confined to those mentioned above but, apparently, the division and accompanying phenomena can be brought about by means of threads dipped in all bases which form soaps with fatty acids. Thus tenth-normal potassium hydroxide or sodium hydroxide and a saturated solution of calcium hydroxide, all bring about the division, although the division when calcium hydroxide is used is less rapid than when tenth-normal sodium or potassium hydroxide are employed, because the concentration of a saturated solution of calcium hydroxide is only about twentieth normal. The division and accompanying phenomena are also elicited in a marked degree by threads dipped in **Choline**.

Not only the bases, but the soaps themselves bring about the division; thus if a thread smeared with **Choline Oleate** be laid across the diameter of a drop of olive oil, the division of the drop will occur, although more slowly than when choline itself is used. This shows that the action of these bases is due to the soap which is formed when they come into contact with the oil and not to hydroxyl ions.

Now we have seen that the phosphoric acid component of the nucleic acid molecule is probably derived, during nuclear synthesis, from **Lecithin** or similar **Phospholipins**. The decomposition of lecithin for this purpose must lead to the setting free either of **Choline** itself

or of a soap of choline or some other nitrogenous base, formed by combination with the fatty-acid radicals of the phospholipin. Immediately following the division of the cell-nucleus into two, which precedes by a definite interval the division of the cell, we may suppose an active synthesis of nuclear materials to be occurring in the two nuclear regions. Hence, in these localities, provided that the above hypothesis be correct choline or some other nitrogenous base would be set free. If now, choline be liberated at both nuclei and diffuses from each nucleus equally in all directions its maximal concentration must obviously occur in the equatorial plane at right angles to the line joining the two nuclei. We have seen that choline, when applied to the diameter of a droplet of liquid immiscible with water (provided soap is formed) results in the division of the drop along that diameter. It is possible that choline, set free in nuclein synthesis, brings about, in a similar manner, the division of the cell, through the formation of soaps in the equatorial plane, either through combination with fatty acids in the cytoplasm, or else through its having been liberated in the neighborhood of the nuclei in combination with one or more of the oleic, stearic or palmitic acid groups of the lecithin molecule.

It is not even necessary to presuppose an actual separation of the two nuclei; it is only necessary to suppose that the nuclein synthesis occurs with greater rapidity at opposite poles than elsewhere within the nucleus in order to understand how nuclear division may be brought about by essentially the same mechanism as that which brings about cell-division itself.

ARTIFICIAL TWIN-FORMATION AND THE FORMATION OF MONSTROSITIES.

In the normal development of the egg the early cleavage-cells, although distinct and separated from one another by a definite interface, nevertheless remain in close apposition to one another, So long as this is the case a single embryo develops. If, however, the first two cleavage-cells chance to fall apart and cease to remain in their normal closeness of apposition then each of the cells develops into a separate and complete embryo and twins are formed from a single egg; these are probably similar in origin to the "identical twins" which are occasionally encountered among higher animals and man.

It has been found by Loeb that the separation of the first cleavage-cells may be brought about in over ninety per cent. of fertilized sea-urchin eggs, provided they are merely exposed, for some time after the first cell-division, to an artificial sea-water differing from normal sea-water in the lack of any one of the constituents **Sodium**, **Potassium** or **Calcium**. This change in the composition of the surrounding saline medium apparently so alters the consistency of the surfaces of the cleavage-cells that they no longer adhere to one another. It may be noted that as the fertilization membrane still surrounds both of the cleavage-cells and the composition of the external saline mixture can nevertheless affect the surfaces of the eggs, the fertilization membrane

must be freely permeable for inorganic salts, although, as we have seen, it is not permeable for colloids.

The two embryos develop side by side within the fertilization-membrane and form swimming blastulæ. At the usual time the membrane bursts and sets the free-swimming embryos at liberty. They are smaller than normal embryos of the same age but otherwise differ in no respect from embryos which arise in the usual way.

The opposite phenomenon, that of fusion of two egg-cells may also be brought about in a certain percentage of cases by treatment of the eggs with alkaline sea-water. This results in the production of gigantic embryos. Even at later stages of development similar fusions may be made to occur. Thus Stockard has found that fusion of the cells which subsequently give rise to the eyes of a fish embryo, *Fundulus heteroclitus*, may be caused by immersing the embryos at a certain stage of their development in sea-water containing an excess of **Magnesium**. The effect of this is to cause the development of fishes provided only with a single cyclopean eye. The origin of these and other like phenomena is to be sought in the influence which the composition of the surrounding medium exerts upon the consistency of the protein and lipoid emulsions within and at the surfaces of the cells.

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CHAPTER XX.

PROCESSES INFERRED FROM INDIRECT OBSERVATION: GROWTH.

GENERAL CHARACTERISTICS OF THE GROWTH-PROCESS.

Regarded from the chemical point of view the growth of animals consists, essentially, in the transformation of simple, unorganised **Foodstuffs**, such as water, the inorganic salts, fats, carbohydrates, amino-acids, and so forth into new chemical entities which, collectively regarded, form the organised protoplasm of the animal tissues. Growth, therefore, involves the synthesis of a variety of chemical compounds in due proportion and succession to one another.

This process obviously does not take place with uniform velocity throughout life. It is not at all unusual, for example, for an infant to grow, during the first months succeeding birth, at the rate of two pounds per month. Were this rate of growth maintained, then at twenty years of age we would weigh in the neighborhood of five hundred pounds.

Nevertheless the process of growth is not one which undergoes a uniform retardation, diminishing in velocity by a uniform proportion per annum. On the contrary, the growth of children, and of animals, takes place in spurts, separated more or less distinctly from one another by periods of relatively languid growth. Thus the rate of growth *in utero* during the first half of gestation is so slow that prior to this period the weight of the human fœtus is inappreciable in comparison with that of the mother. This period of slow growth is succeeded by the extraordinarily rapid accretion of tissue which characterises development during the months immediately prior to and succeeding delivery. A definite slackening of growth occurs, however, toward the end of the first year of extrauterine life, and this slowing down of growth is not an artefact, dependent upon weaning, since it occurs just as strikingly in bottle-fed infants. This resting period is succeeded by the relatively rapid growth of the third, fourth, and fifth years Another pause or slackening of growth succeeds this, to be followed by the energetic growth which accompanies adolescence.

The growth of man, therefore, consists of periods of rapid and slow growth which alternate with one another, and if we plot the growth in any dimension, for example the growth in *weight*, on "coördinate paper" so that the weights are measured vertically and ages horizontally, we obtain a diagrammatic picture of the growth-process which is not a

straight line, nor even a single curvilinear sweep, like the outline of a parabola or of the logarithmic curve which represents the progress of the ordinary type of chemical reaction. On the contrary, our diagram reveals distinct waves or large oscillations in the growth-process and resembles, as a matter of fact, the diagram which may be obtained by superimposing three S-shaped curves upon one another in such a manner that their adjacent extremities merge into one another.

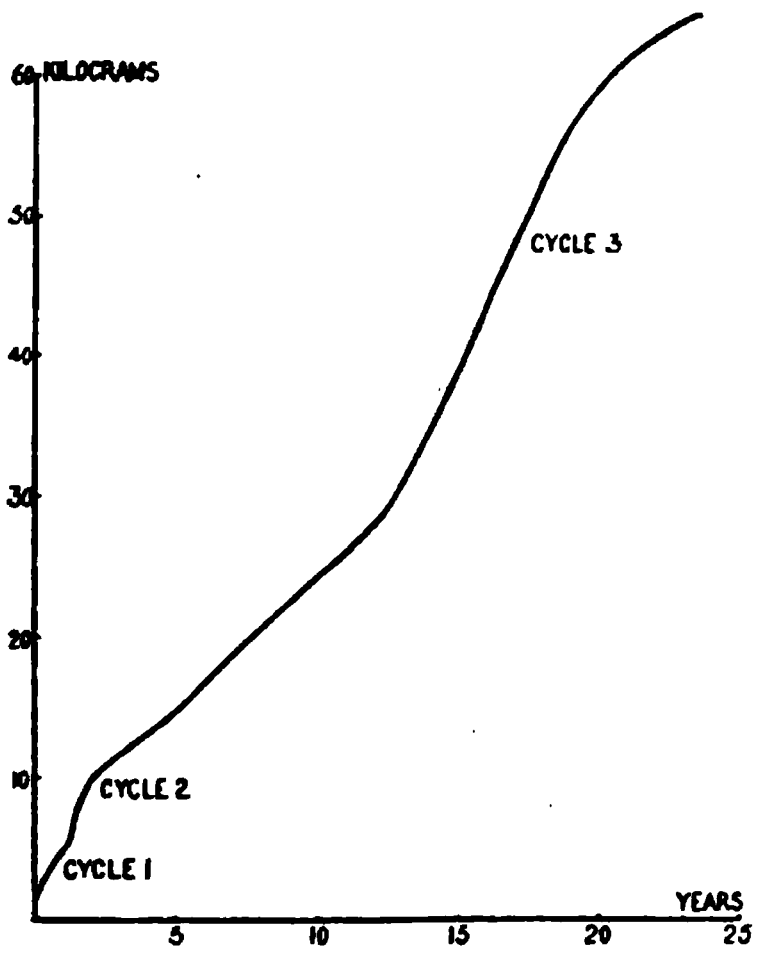
These waves or oscillations, or "**Growth-cycles**," as we may term them, are not accidental. They are easily distinguishable from the relatively slight irregularities or fluctuations of growth which every individual child or animal will display more or less frequently during its development. They are distinguishable from such accidental fluctuations because they occur at very nearly the same places in the growth-curve of every normal child, and in the average growth-diagram constructed from the data supplied by a large number of individuals, these large oscillations reveal themselves very distinctly, while the accidental and individual fluctuations cancel out and disappear in the average diagram because, in the long run, if we take a sufficient number and variety of individuals into account, just as many of these accidental fluctuations will be positive (*i. e.*, supernormal in weight) as negative (*i. e.*, subnormal in weight). But the large fluctuations, or Growth-cycles, remain unaffected in magnitude and position, and only appear more definitely in the diagram the greater the number of individuals which we measure or weigh.

In the **Growth of Man** there are, in all, three distinguishable growth-cycles which are superimposed upon one another. Each cycle begins with a period of relatively slow growth, followed by a period of very rapid growth, and culminating, with the termination of the cycle, in a period of slackening growth again. In the case of the first two cycles this slackening of growth is followed by the fresh spurt or acceleration due to the succeeding cycle. In the case of the third or adolescent cycle of growth, the period of slackened growth-velocity insensibly merges into the period of relatively stationary development which we recognize as the adult condition. This developmental stasis may be interrupted, however, by the repair incident to the replacement of tissue which has been injured or destroyed, while even in the absence of such **Regenerative Growth** a vigorous and abnormal growth may occur, the growth, namely, of **Malignant Tumors**, which we may possibly interpret as constituting the superposition of a fourth, and physiologically abnormal cycle of growth upon the third and normally final cycle in the development of man.

Not only the growth of man, but also the growth of every mammal which as yet has been carefully investigated appears to consist of three more or less easily distinguishable cycles of growth. The growth of the **Guinea-pig** at first appeared to consist of only two difficultly distinguishable cycles, but the investigations of Read have shown that in this mammal the first growth-cycle is actually completed *in utero*,

instead of being interrupted when half-completed by birth, as it is in human beings. Corresponding to this we find that the guinea-pig is born at a much more advanced stage of development than man or the rat or mouse; their eyes are open, they have a full coat of hair, are able to choose and eat their own food and may be weaned altogether within a few days after delivery. The very general occurrence of *three* growth cycles in mammalian development renders very inviting the supposition that they are referable to the existence of *three* **Embryonic Layers**, from one or other of which all the tissues of the adult are ultimately derived, but for this hypothesis there are as yet lacking the necessary experimental and anatomical proofs.

In the accompanying figure (Fig. 31) are compared the growth-diagrams of human males of British birth and parentage and of male white mice. The resemblance between the two curves, allowing for the difference of the time-units employed, is of a very striking character. The only notable difference lies in the relatively marked delay of the third, or adolescent growth-cycle in man as compared with the mouse, the possible origin of which will be discussed subsequently.



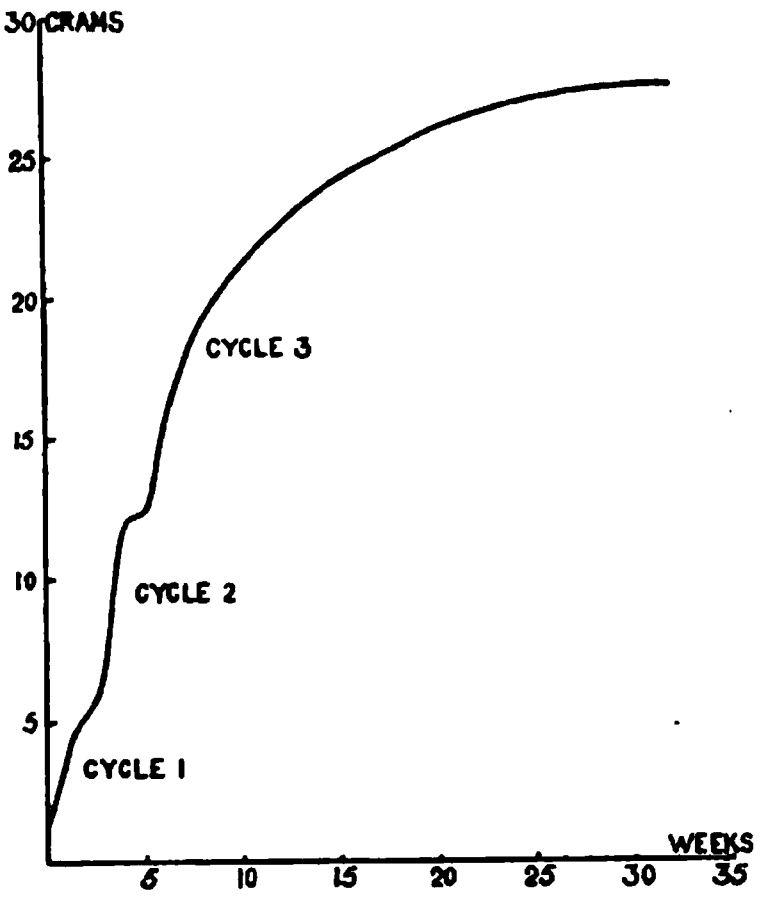


FIG. 31.—Growth of human males. (Constructed from the data obtained by the British Anthropometric Committee.)

Growth of male white mice.

These **Growth-cycles**, so definitely situated in the curve of growth, and so invariable in their occurrence that they may be clearly recognised in the growth of mice no less than in the growth of man, must have some very definite physiological significance, and since, as we have seen, growth is essentially a chemical process resulting in the synthesis of living tissue from inanimate materials, these growth-cycles must have a chemical, no less than a physiological significance. The general similarity of the fundamental phenomena of growth in all living forms

is strikingly revealed by the fact that the curves of growth obtained in **Plants** and even in the multiplication of **Bacteria** are essentially similar in character to those obtained in animals. As a rule, however, the growth of a plant or of a colony of bacteria displays evidence of only a single growth-cycle.

Each of these growth-cycles is approximately symmetrical about its center, that is, on either side of the moment of most rapid growth; in other words the second half of the S-curve reproduces in the reverse order the characteristics of the first half. We have, then, in each growth-cycle considered by itself, a chemical process which begins relatively slowly, increases progressively in velocity until it is about half completed, and then slows off to its termination. The inquiry now immediately presents itself whether any chemical processes of

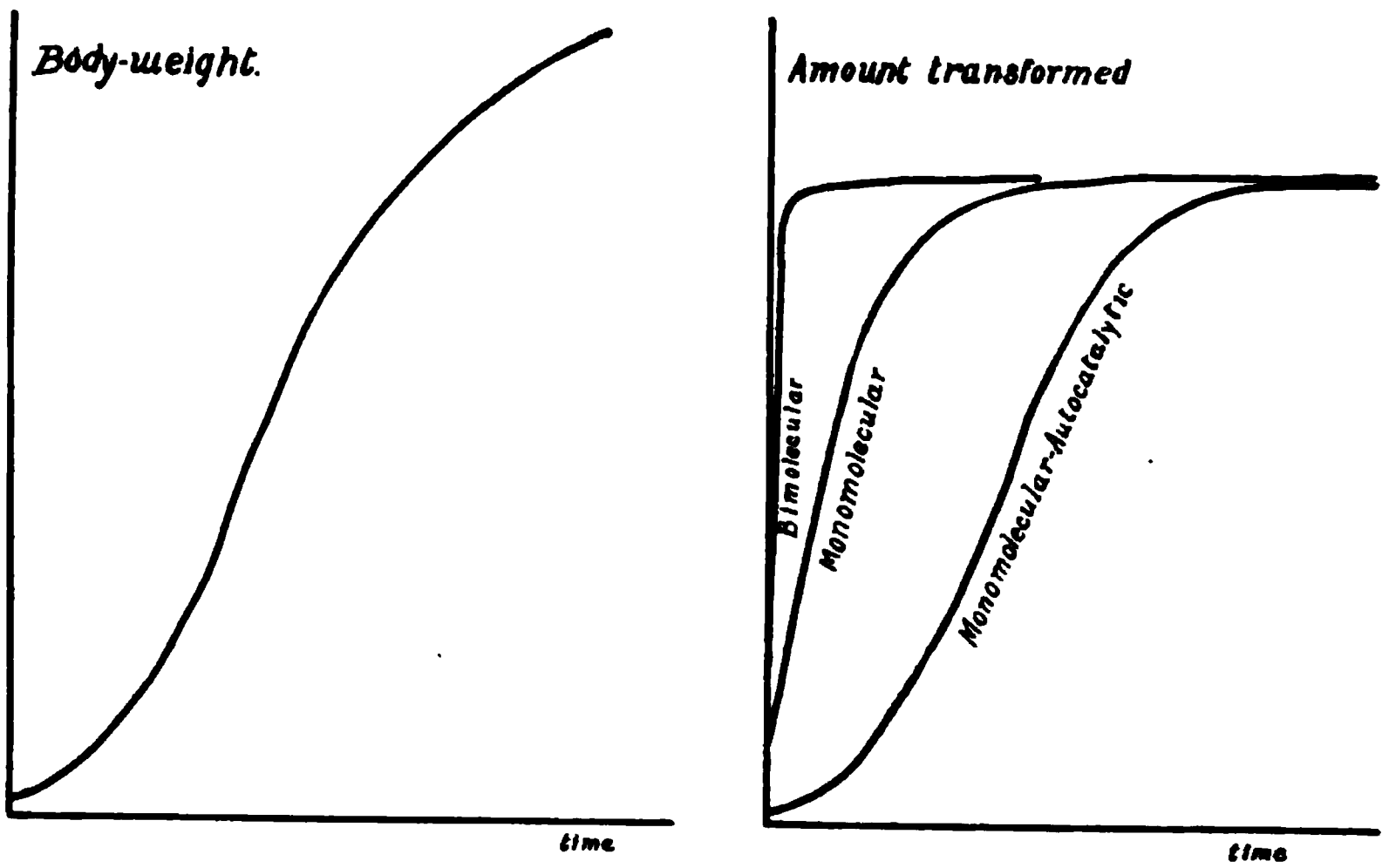


FIG. 32—Comparison of the curve of growth of the white rat (constructed from data collected by Donaldson) with chemical reaction curves.

this general character are known to occur elsewhere than in the building up of tissue by a growing plant or animal? As a matter of fact, chemical transformations of this character are abundant and they are those in which one or more of the products catalyzes the further progress of the reaction. We have already in preceding chapters had occasion to dwell upon a number of chemical phenomena which occur in living tissues and elsewhere which belong to this category; it will merely be necessary therefore to refer in passing to the analogies afforded by the hydrolysis of castor-oil in the seeds of *Ricinus*, the hydrolysis of cane-sugar by boiling neutral water, the decomposition of methyl acetate by water, the oxidation of metals and of a variety of organic materials, and the chemical transformations which accompany and underlie the performance of muscular work. In all of these various

processes one or more of the products of the reaction is endowed with the property of facilitating the further progress of the reaction. Such transformations are designated **Autocatalyzed Reactions** (Fig. 32).

The fact that each growth-cycle begins slowly and progressively increases in velocity until the moment of maximal growth-velocity is attained at the center of the cycle is sufficient in itself to show that the process of growth is autocatalyzed, whatever the mechanism of the self-acceleration may be. The resemblance of the process of growth to the transformations in an autocatalyzed reaction is not merely superficial, however, but extends even to quantitative details.

It will be recollected that the relationship between the extent of transformation and the time in an ordinary **Monomolecular Chemical Reaction** is expressed by the equation:

$$\text{Velocity} = k(a - x)$$

where "a−x" is the amount of the original material which is as yet untransformed and "k" is a constant, specific for the particular reactions under consideration. The effect of catalyzers upon such a reaction is to multiply the value of "k" by a quantity which is proportional to the amount of catalyzer present. Now in an autocatalyzed reaction the amount of catalyzer which is present is proportional to the mass of the product of the reaction, that is, to "x." The equation for an **Autocatalyzed Monomolecular Reaction** becomes, therefore:

$$\text{Velocity} = kx(a - x)$$

which, when integrated, yields the equation:

$$\log_{10} \frac{x}{a - x} = ka(t - t_1)$$

where t is the time from the beginning of the measurements and t_1 is the time at which the reaction is half completed, *i. e.*, the center of the autocatalytic curve.

The applicability of this equation to the growth in numbers of **Bacteria** in a limited quantity of culture medium has been established by McKendrick. It is, however, not less applicable to relatively complex phenomena of **Human Growth**. The juvenile and adolescent cycles of growth in man are rather closely interfused, so that their separation into individual cycles is a difficult and uncertain matter. The infantile cycle, however, is rather definitely separated from the remainder of the human-growth curve by a rather long period or "plateau" of relatively slow growth. The **Infantile Growth-cycle**, therefore, at any rate for the first ten months succeeding birth, presents the relatively uncomplicated characteristics of a single cycle of growth to which the above equations may be applied. In the following comparisons of the theoretical values calculated from the equation

$$\log_{10} \frac{x}{a - x} = ka(t - t_1)$$

with the average values actually obtained from weighings of large numbers of infants the constants "a," "k" and "t," are calculated from all of the observations by the "method of least squares. In this way, for example, we find that the growth of British male infants born in South Australia, during the first nine months succeeding delivery, is expressed by the formula

$$\log_{10} \frac{x}{341.5 - x} = 0.136(t - 1.66)$$

time being reckoned in months from birth and weights in ounces avoirdupois. In the following table the observed weights at the various ages are compared with those calculated from this formula:

SOUTH AUSTRALIAN MALES.

Age of infant in months.	Weight in ounces.	
	Observed.	Calculated.
0	127	127
1	155	156
2	187	180
3	206	206
4	224	230
5	254	254
6	270	273
7	287	288
8	300	301
9	311	311

The equation to the curve of growth for the first nine months of the extra-uterine life of South Australian females is found to be:

$$\log_{10} \frac{x}{350 - x} = 0.111(t - 2.47)$$

and in the following table the observed weights at various ages are compared with those calculated from the formula:

SOUTH AUSTRALIAN FEMALES.

Age of infant in months.	Weight in ounces.	
	Observed.	Calculated.
0	121	121
1	153	142
2	168	164
3	188	187
4	209	209
5	224	230
6	253	249
7	263	267
8	270	282
9	300	295

A similar comparison follows for British infants born in England: The equation to the infantile growth-cycle during the first nine months in males is represented by the formula:

$$\log_{10} \frac{x}{318 - x} = 0.127(t - 1.46)$$

BRITISH MALES.

Age of infant in months.	Weight in ounces.	
	Observed.	Calculated.
1	147	148
2	169	171
3	194	194
4	219	216
5	234	235
6	252	252
7	269	266
8	276	277
9	283	287

The equation for the same period in females is represented by:

$$\log_{10} \frac{x}{312 - x} = 0.106(t - 1.54)$$

BRITISH FEMALES.

Age of infant in months.	Weight in ounces.	
	Observed.	Calculated.
1	143	146
2	160	165
3	180	184
4	202	202
5	218	218
6	235	233
7	253	247
8	258	259
9	265	269

In all cases it will be seen that the agreement between the observed and the calculated weights is extremely close; in fact such consonance between the quantitative demands of a theoretical equation and the experimental estimations is not frequently obtained even in experiments conducted in laboratory-glassware. The probable reasons for the extreme regularity observed lie in the first place in the large number of measurements from which each average weight is computed and in the second place in the excellent conditions of thermostasis which the body of a warm-blooded animal affords.

Even in such complex *Metazoa* as man, therefore, the process of growth in an individual growth-cycle appears to be determined and governed by the simple law which is characteristic of an **Autocatalyzed Monomolecular Reaction.** It will at once occur to the reader, however, that the process of growth, taken as a whole, cannot possibly be of this simplicity, for in the construction of the simplest of the multitudinous constituents of tissues a variety of parallel and successive chemical reactions must as a rule contribute to the result. The diversity of interdependent chemical phenomena involved in the building up of an organism so complicated as ourselves must be almost unimaginably great. How, then, can a reaction-formula characteristic of a single and uncomplicated transformation, peculiar only in producing its own catalyzer, apply to the quantitative outcome of such a bewildering tissue of chemical events?

The answer to this question is undoubtedly to be sought in the fact that in any system of interdependent chemical transformations the *slowest reaction in the series governs the velocity of the whole.* On the hither side of the slowest reaction all the raw materials for subsequent processes must accumulate and await the elaboration of the products which they utilize, while on the far side of the slowest reaction the subsequent processes are retarded to its pace by the consumption of their substrates. The slowest reaction in any chain of chemical processes is the **Master-reaction** which determines from moment to moment the quantitative relations of the product to the time. Now in the complex of events which constitutes growth not a single significant transformation is independent of the rest; each must evidently use some product of other transformations and contribute some product to get another series of processes. We can therefore understand how the whole phenomenon, notwithstanding its complexity and the multiplicity of the chemical reactions involved in it, may nevertheless be governed, as to its quantitative outcome, by the rate at which a single reaction occurs. This reaction, as we have seen, is autocatalytic.

We are thus led to inquire whether the growth-diagram, which is so similar in form to the curve which represents the progress of an autocatalyzed chemical reaction, may properly be regarded as establishing the existence of **Catalyzers of Growth** which are numbered among the products of the growth-process, or **Endogenous Catalyzers**, as Hopkins has termed them, and also the existence of **Impeding Factors**, attributable either to the exhaustion of an essential constituent of the reaction, or to the accumulation of growth-products.

The problem becomes somewhat clearer when we consider the simple case of **Bacteria**, growing on a limited amount of a given culture-medium. In this case, as McKendrick has shown, precisely analogous phenomena are exhibited to those which characterize the growth of higher organisms. The growth of the bacterial culture, measured by the total mass or number of bacteria produced at given time-intervals, is at first extremely slow; it increases in velocity, however, and at first almost in proportion to the number of bacteria produced. At a later stage growth is impeded and finally comes to a standstill when the density of the population of the culture-medium has attained a certain maximum.

These phenomena are interpreted by McKendrick in the following manner: Each bacterium is capable of giving rise to a certain number of daughter-cells in a certain interval of time under constant nutritive conditions. This potentiality is transmitted to its offspring, so that were the nutritive constituents of the culture-medium inexhaustible, the velocity of reproduction would always be proportionate to the number of bacteria previously produced, or, in other words, the density of the bacterial population would increase in geometrical, while the time increased in arithmetical progression. In practice, however, the ability of the culture-medium to supply nutritive materials to the

bacteria is limited, and the rate of multiplication is slowed. McKendrick infers, therefore, that the rate of multiplication is proportional to two factors; in the first place to the number of bacteria previously produced, and in the second to the concentration of the still-available foodstuffs. This leads to the equation:

$$\frac{dx}{dt} = kx(a - x)$$

where "x" is the number of bacteria per unit-volume, "a−x" is proportional to the concentration of available nutrients and "k" is a constant proportionality-factor. Integration of this differential equation leads to the relationship

$$\log \frac{x}{a - x} = ka(t - t_1)$$

where "x" is the number of bacteria per unit volume, a is the maximal density of population which is attainable in a given culture medium, "k" is a constant proportionality-factor and t_1 is the time at which the density of the bacterial population has attained half its maximum.[1]

The relationship between the number or mass of bacteria produced and the time of incubation which is expressed in these equations is, however, identical with that which expresses the relationship between weight and age in any given growth-cycle of an animal or plant. It is also identical with the relationship between the mass of the products and the time in autocatalyzed chemical reactions, such as the hydrolysis of **Methyl Acetate**. The question therefore presents itself, whether the process of growth in a multicellular organism such as a mammal is comparable to an autocatalyzed chemical reaction, or whether McKendrick's interpretation of the growth-curve of a bacterial population does not offer an alternative explanation of the facts. In other words two alternative possibilities would appear to exist: the one that the accelerative factor in growth is a chemical substance, as it is in autocatalyzed chemical reactions, the other that it is simply due to the multiplication of cells, each of which is possessed of like potentialities of reproduction.

On closer analysis it will be seen, however, that these interpretations, at first sight alternative, are in reality identical.

Reverting to the case afforded by the multiplication of bacteria in a limited amount of culture-medium, and looking to the beginning and end of the process, we see that the increase in bacterial population means essentially that the simple, unorganized constitutents of the culture-medium have been transformed into the substances composing the bacteria. Any acceleration experienced by the process must ultimately be due to the preceding synthesis, irrespective of the fact that the synthesis takes place in a heterogeneous system, *i. e.*, in the separate particulate masses which form the individual bacteria. When

[1] I have slightly, but unessentially, modified McKendrick's formulation of this relationship in order to make clearer the analogies which follow.

we say that each bacterium has a like potentiality of reproduction we clearly express the fact that the synthesis of bacterial cell-substances which results in the production of a cell is a favoring condition for the production of new cells, in other words that some substance or substances comprising the bacterium accelerate the production of new masses of bacterial substance. In ultimate terms, therefore, the two interpretations of the phenomenon are identical, the only essential difference between the more familiar cases of autocatalysis, such as the hydrolysis of methyl acetate, and the process of cell-multiplication, being the fact that in the latter process the reaction takes place in a heterogeneous chemical system, *i. e.*, within the particulate masses comprising the cells. Yet the fact that a chemical reaction takes place in a heterogeneous medium does not imply that it is discontinuous. The production of calcium sulphate from a mixture of calcium hydrate and sulphuric acid is a continuous process despite the fact that the product is divided into particulate masses, which in this instance are crystals. On the other hand the instances of autocatalysis in heterogeneous systems are abundant in chemical literature, the oxidation of metals in contact with air being a familiar illustration of a group of autocatalyzed reactions of this type.

The **Accelerative Factor** in the process of growth is, therefore, a chemical substance or substances, or a chemical condition, which is strictly analogous to the accelerative factor in less complex autocatalyzed phenomena. The autocatalytic character of the growth-process follows of necessity, in fact, from the fundamental characteristic which, more than any other, distinguishes living from non-living material, namely its potentiality of unlimited *reproduction*. When we assert that living cells all possess like potentiality of reproduction we merely state in morphological terminology that the production of living matter is a self-sustained or autocatalyzed phenomenon. Just as the production of living from inanimate matter is essentially a chemical process, so the acceleration of its production which is consequent upon the multiplication of the particulate resultants of the process is, when viewed from the chemical standpoint, evidence that substances are produced in the creation of living matter which have the essential property of catalyzing its further manufacture. Regarding the possible nature of these endogenous catalyzers, we shall have something to say in a later part of this chapter.

It remains to consider what may be the probable nature of the **Inhibitive Factor** which ultimately brings the process of growth to a standstill, which sets a limit to the normal dimensions of any given species of animal, and which predominates over the accelerative factor during the latter half of each growth-cycle. In the simpler instances of autocatalysis, as we have seen, the inhibitive factor may be, either the exhaustion of the materials undergoing transformation, or, on the other hand, the accumulation and consequent "back-pressure" of the products of the reaction, or both of these factors may play a part in

determining the magnitude of the inhibition. Either of these alternatives would yield the time-relations expressed in the autocatalyzed reaction-formula, for the following reasons:

In case the velocity of the reverse reaction is, at all stages of the transformation, negligible in comparison with that of the forward reaction, then the only inhibitive factor must be the exhaustion of the **Substrate**, or material undergoing transformation. The velocity of the process will be, as usual in chemical reactions, proportional to the mass of untransformed material and also to the mass of the catalyzer, that is, in these instances, to the mass of the products of the reactions. Designating the mass of a product of the reaction at any moment by "x," and "a" the initial amount of the material undergoing transformation, this yields the relation:

$$\text{Velocity of transformation} = \frac{dx}{dt} = kx(a - x)$$

which is the formula characteristic of an autocatalyzed reaction.

Coming, now, to the case in which the velocity of the reverse reaction is so considerable as to be comparable with that of the forward reaction, we will assume, in the first instance, that the materials undergoing transformation (or foodstuffs in growth) are inexhaustible, *i. e.*, are constantly being renewed from the environment, so that the mass of material undergoing transformation is a constant which we may designate by the symbol of "A." The velocity of the forward reaction will then be, as in the above instance, proportionate to the mass of the catalyzer (=product of the reaction="x") and also to the constant mass of substrate, that is, to "A." The velocity of the reverse reaction (breaking-down of the products of the reaction into the initial substances again) will be proportional to the mass of the products (="x"), but also to the mass of the catalyzer (="x"), because in the majority of instances of "typical" catalysis the catalyzer accelerates both the forward and the reverse reactions in equal proportion. The velocity of the reverse reaction at any moment will therefore be proportionate to x^2, and the net velocity of the process, being the difference between the velocities of the forward and the reverse reactions, will be given by:

$$\frac{dx}{dt} = k_1 x A = k_2 X^2$$

in which "k_1" and "k_2" are the velocity-proportionality factors of the forward and reverse reactions respectively. Rearranging the terms of the equation this may be written:

$$\frac{dx}{dt} = k_2 x \left(\frac{k_1}{k_2} A - x\right)$$

which is again identical with the ordinary formula of autocatalysis, with the exception that the constant "a," denoting the maximal attainable value of "x" is now not the initial mass of material undergoing

transformation, but the initial mass multiplied by the constant ratio of the velocity-constants of the forward and reverse reactions.

In the case of the growth of **Bacteria** in a limited quantity of culture medium, McKendrick assumes that the inhibitive factor is simply the exhaustion of available foodstuffs, *i. e.*, that it corresponds to the first of the alternative possibilities outlined above. In the growth of animals, however, it is difficult to see how the limited availability of **Foodstuffs** could be a deciding factor in the inhibition of normal growth, for the medium in which our cells actually live and grow is the lymph (or "tissue-fluid"), which is constantly supplied and renewed from the blood. Now the mechanisms of the body are, as we have seen, so devised that the composition of the blood is maintained in a condition of extraordinary uniformity. It is true that its content of the more particularly nutritional constituents fluctuates with the fluctuating absorption of nutrients from the alimentary canal, but these short-period fluctuations result in the long run in the maintenance of a remarkably steady flow of nutrient materials to the tissues. The blood derives its nutrient constituents from the external environment and in fact contains them not merely in sufficient proportion to maintain an equilibrium of body-weight, but, even in adult animals, in considerable excess of the necessary minimum, the destruction of this excess constituting the "**Exogenous Metabolism**" as contrasted with "**Endogenous Metabolism,**" or irreducible minimum of nutrient-consumption incident to the maintenance of life. The medium in which our cells live, therefore, is under normal dietetic conditions a medium of almost constant composition and, for the purposes of tissue-synthesis, it is inexhaustible since it is continually renewed. The **Substrates** of growth must therefore be regarded as being of constant concentration and the inhibiting factor of growth must be sought elsewhere than in the exhaustion of available nutrients.

On the other hand, if a portion of the tissues of an adult animal be injured or destroyed, the process of growth immediately recommences and is expressed in the phenomenon of **Regeneration** which, if mechanical factors do not impose an insuperable obstacle, continues until the complete restoration of the lost tissues has been accomplished. In other words, removal of the products of growth immediately reinaugurates the growth-process, just as the removal of the products of a "balanced" chemical reaction at equilibrium immediately reinitiates the forward reaction. We must infer, therefore, that in the growth of mammals, at least, it is the accumulation of the **Products of Growth** which normally inhibits the process and not the exhaustion of nutritive materials. In **Plants** the supply of nutritive materials to the cells is more fluctuating and dependent upon the environment, and here we may expect to find, and do actually find, a much more conspicuous part played by the supply of nutrients in determining the final attainable dimensions of the organism. Nevertheless plants of a given species, even under the most favorable nutritional conditions, do not exceed certain definable limits in their dimensions at maturity, and they

display regeneration when portions of their tissues are removed. Even in the case of bacteria growing in a limited supply of culture-medium, there is evidence which tends to show that in many cases the accumulation of bacteria or bacterial products really sets the limit to their multiplication rather than the exhaustion of the nutrients in their culture medium.

We infer, therefore, that the process of growth is governed by a series (in mammals usually three) of autocatalyzed chemical reactions in which the factor which determines the retardation and ultimate equilibrium of the process is the accumulation of the products, *i. e.*, the growth itself.

The constancy of the concentration of **Growth-substrates** in animals affords a readily intelligible explanation of the extraordinary simplicity of the quantitative relationship between growth and time, which, as we have seen, so frequently obtains. The relationship in question is that which characterises the progress of an autocatalyzed monomolecular reaction, and even admitting the probability that a single chemical transformation may determine the speed and set the pace for the whole of the multitudinous variety of chemical processes involved in the growth of new protoplasm, yet it may seem strange that even this single reaction should be of so simple a character, more especially since, as the construction of protoplasm involves synthesis of large out of relatively small molecules, we would expect any reaction involved in growth to be *multimolecular*. Now this may actually be the case, even in the **Master-reaction** which determines the quantitative outcome of all the growth-processes, for if the concentration of the substrates of growth remains undiminished by the growth which occurs, then any number of molecules of the substrates may participate in the synthesis which constitutes the governing reaction, without involving any departure of the relationship between the time and extent of growth from that which is expressed in the monomolecular autocatalytic formula. If "n" molecules of the substrate combine to form one molecule of the product, then the velocity of the forward reaction will be given by:

$$\frac{dx}{dt} = k_1 x A^n$$

while that of the backward reaction will be given, as before, by

$$-\frac{dx}{dt} = k_2 x^2$$

hence the net effect, or actual growth, will be given by

$$\frac{dx}{dt} = k_1 x A^n - k_2 x^2$$

which, rearranging the terms, becomes:

$$\frac{dx}{dt} = k_2 x \left(\frac{k_1}{k_2} A^n - x\right)$$

which is again of the monomolecular form, save that the constant "a" in the formula is no longer proportional to the actual concentration of the substrates, but to the *nth* power of their concentration. That the backward reaction should be monomolecular is, of course, not a matter for surprise, since we may suppose that the majority of decompositions which living tissue suffers consists in the interaction of a single molecule of some protoplasmic constituent either with water or with oxygen, the concentration of both of which substances is maintained automatically at an approximately constant level in the tissues.

Thus the synthesis of a protein involves the interaction of many different amino-acid molecules, but its hydrolysis in dilute aqueous solution obeys the monomolecular formula, because only a single species of molecule, that of the protein itself, is undergoing appreciable change of mass or concentration in the process.

Summarizing the general characteristics of the growth-process we may therefore state:

1. That the growth of man and of animals takes place in periods or cycles in which slow and rapid growth alternate, three of the cycles being usually appreciable in magnitude.
2. Each of the growth-cycles is the expression of an underlying self-accelerated chemical process.
3. The accelerating factor is some substance or group of substances produced during growth.
4. The supply of nutriment capable of transformation into living tissues may, in normal animals, be regarded as constant and undiminished by the process of growth itself.
5. The inhibiting factor, which ultimately brings the growth in any given cycle to a standstill, is the accumulation of the products of growth.
6. Removal of these products, as by local death or injury, or by general inanition, reinaugurates the process of growth, which continues until equilibrium is reattained.
7. The whole of the diverse processes which in the aggregate constitute growth are governed and determined in rate and magnitude by the specificially slowest essential process.
8. The forward reaction in the governing process may involve the interaction of many different molecules, but the reverse reaction appears, in many cases at least, to involve the decomposition of only a single species of molecule of variable mass or concentration.

THE INFLUENCE OF RACE, SEX, AND ENVIRONMENT UPON THE GROWTH-PROCESS.

The fact that the bodily dimensions of a given species of animal never exceed certain characteristic upper limits, no matter how favorable the environmental conditions may be with respect to the abundance and variety of **Nutrients**, shows that the factors which inhibit the

growth in any given growth-cycle are primarily characteristic of the process itself and only in a minor degree dependent upon the dietary, provided it is in all respects sufficient. We have seen that the main inhibiting factor in growth arises from the accumulation of the products of growth and the enhanced rapidity of tissue-disintegration which ensues. The characteristic dimensions of an animal, therefore, and the same, to a less striking degree, is doubtless true of a plant, are determined mainly by the relative magnitude of the specific **Velocity-constants** of the forward and the opposed reactions. These are characteristic of the particular reactions which occur in a given race or sex, and are not influenced by the mere abundance or paucity of the dietary.

That the bodily dimensions of an animal may be affected to a limited extent by the abundance of the **Dietary** is, however, a readily ascertainable fact. If the dietary be absolutely insufficient even to maintain bodily heat and the output of work, the tissues are called upon to supply the energy-requirements, the animal loses weight and may ultimately die of inanition or of acute conditions supervening upon partial inanition. If the dietary insufficiency is less extreme than this, growth is nevertheless slowed, and the bodily dimensions attainable at maturity are smaller than is normal for the species. If, on the other hand, the diet is exceedingly abundant and other environmental conditions are exceptionally favorable, then the bodily dimensions at maturity may come to distinctly exceed the average, although the degree of supernormality which is attainable in this way is, of course, strictly limited. Mice, under no matter what favorable conditions of environment and abundance of food supplies, do not achieve the bodily dimensions of a guinea-pig or even of a rat.

The supply of nutrients to the tissues is, as we have seen, determined primarily by the composition of the blood which, subject to short-period fluctuations, remains relatively constant throughout the growth and life of the animal. The "**Nutrient Level**" or concentration of growth-substrates in the blood is maintained by a dynamic equilibrium which involves a variety of factors. On the one hand we have the availability of **Foodstuffs** in the external environment and the ability of the digestive apparatus to disintegrate them and to absorb the products of their disintegration. On the other hand we have the rate of utilization by the tissues and the equilibrium between the storage-capacities of the tissues for the various classes of foodstuffs, for polysaccharides, fats, and amino-acids, and the concentration of these substances or their products in the blood and tissue-fluids. The height of the nutrient reservoir in the blood is thus governed by a balance between a certain rate of *inflow* and a certain rate of *outflow*. In addition to these factors, and in order to avoid an excessive accumulation of nutrient materials in the blood, an *overflow* is also provided in the phenomenon of **Exogenous Metabolism**, or the destruction of food-

stuffs which have not yet come to comprise living matter, a process which, in the case of the amino-acids at all events, forms a very large proportion of the total metabolism of a normally nourished animal.

If any of these several factors is decidedly altered in magnitude or velocity a more or less marked effect upon bodily weight will ensue. Thus if the rate of *inflow* be diminished beyond a certain point by an insufficient dietary the nutrient level sinks and growth is retarded, or, in the adult animal which has attained growth-equilibrium, the process of growth may be reversed and loss of tissue occur. The extent of this reversion is strictly limited in the more complex forms of *metazoa* by the necessity of maintaining certain mechanical conditions: the integrity of the skeleton, the functional ability of the digestive organs, the pulsation of the heart, the integrity of a closed vascular system, the coördinating activities of the nervous system, and the continuance of respiratory movements. If any of these suffer in so complex an organization as our own the whole must fail and death ensue. But in some less complex forms, as in the fresh water worm *Planaria*, starvation actually accomplishes **Reversion of Growth** until an embryonic stage of development is regained (Child).

If, on the other hand, the rate of inflow of nutrients be maintained unaltered and the rate of *outflow* increased or diminished the rate of accretion of tissue must obviously be affected to a proportionate degree. In normal cases, since the rate of outflow or consumption of nutrients for tissue-building purposes is determined by the relative magnitudes of the specific velocity-constants of upbuilding and disintegration, the rate of outflow will vary in different species and not improbably in the two sexes of the same species, and to a certain extent in different individuals. The environment, on the contrary, provided the inflow of nutrients is maximal or at least sufficient, may be expected to play little if any part in determining the rate of outflow.

The rate of *overflow* is also conditioned primarily by internal regulation, but we may observe the effects of its alteration in so far as the nutrient-level of the amino-acids is concerned, by the pronounced effects of hyper- or hypo-activity of the **Thyroid** upon the development of the tissues. The administration of thyroid extract leads to a very decisive increase in the rate of **Deaminization** of amino-acids, and in normal adults who have attained growth-equilibrium, this, which involves a fall of the nutrient-level, results in progressive loss of weight which may, if it affects essential tissues, result in dangerous or even fatal symptoms. The effects of hypo-activity are the opposite and the excessive accretions of tissue not being uniformly distributed, aberrations of growth occur which culminate in the condition of **Myxedema**. In amphibians excision of the thyroid, as Gudernatsch has very strikingly demonstrated, results in the arrest of **Metamorphosis**, possibly because the degeneration of certain tissues which is a necessary precedent of metamorphosis cannot occur.

In the autocatalytic formula as applied to the process of growth:

$$\log \frac{x}{a - x} = ka(t - t_1)$$

the constant "a" is proportional to some exponent of the concentration of growth-substrates, *i. e.*, to the **Nutrient-level.** In any given species, therefore, we may expect to find that within certain limits its magnitude is affected by the environment and especially by the abundance or paucity of the dietary. The constant "k" on the contrary is expressive of the specific velocity of the process of tissue-disintegration, characteristic of the species, probably of the sex, and peculiar even to a particular individual. Thus we may expect, in a given species, to find that its magnitude is unaffected by the environment, but dependent upon **Sex and Race.** We have seen that the autocatalytic formula applies to the first nine months of extra-uterine growth in infants and that the values of "a" and "k" may be computed from all of the observed weights at the various ages chosen for the comparison of the equation with the results of actual measurement. In the following table the values of "a" and "k" for British Infants born in England and in Australia respectively and for South German infants born in Frankfurt (from the data of Schmidt-Monnard) are compared:

COMPARISON OF THE EFFECTS OF RACE AND ENVIRONMENT UPON THE PARAMETERS OF THE GROWTH-CURVE.

Race and place of birth.	Males.		Females.	
	a (ounces).	kx10⁶.	a (ounces).	kx10⁶.
British (born in England) . .	318	399	312	340
British (born in Australia) . .	341.5	398	350	317
South German	315	451	290	537

It will be seen that the parameters or constants of the growth curve of infants are affected in the sense indicated by the above discussion by the factors of sex, race and environment. While the value of "a" is not greatly affected by sex or by dissimilarity of race, the values obtained in the similar environments of Frankfurt and London being very alike, it is greatly affected by dissimilarities in environment, as a comparison of the values of "a" in Australia and in Europe shows. On the other hand, "k" is comparatively unaffected by environment, being practically identical for British males, whether born in Australia or in England, and very nearly the same for British females born in these two environments, whereas it is profoundly affected in magnitude by sex and race, as indicated by the marked difference in the values of "k" for males and females and for South-German as compared with British infants.

When it is remembered that these parameters have not been calculated arbitrarily, but that they are computed by the method of least squares from all of the observations and therefore partake in some measure in the errors incident to the observations, it will be seen that

the above data afford a very remarkable demonstration of the correctness of the view that growth is determined by an underlying autocatalyzed chemical process. It is furthermore clear that the form of the curve of growth in normal infants is determined by two separate groups of factors. The one, analogous to the absolute mass of the reacting substances in a chemical reaction, being dependent upon the environment and probably largely influenced by the abundance or deficiency of the habitual dietary; while the other, analogous to the specific velocity of a chemical reaction, is relatively, if not absolutely, independent of environmental or nutritional conditions, and, being expressive of the nature of the growth-process itself as distinguished from the availability of the materials for growth, is distinctively modified by race and sex.

THE SUBSTRATES OF GROWTH.

The substrates of growth, *i. e.*, the material out of which living tissues are synthesized, are the **Foodstuffs**, namely oxygen, water, inorganic salts, carbohydrates, fats and proteins. In the period of biochemical research which immediately followed the fundamental discoveries of Liebig and Voit, the application of the laws of the conservation of matter and energy to the phenomena of growth and metabolism appeared to supply all of the necessary clues for the interpretation of the relationship of the foodstuffs to the maintenance of life. But with the increasing refinement of our knowledge of the intimate chemical structure of the foodstuffs themselves it has become increasingly apparent to us during the recent decades that it is not sufficient merely to supply an animal or a human being with a sufficiency of nitrogen, carbon and calories to replace his daily waste in order to maintain the equilibrium between waste and repair in his tissues, nor is it even sufficient to supply these desiderata in digestible and assimilable form; it is furthermore necessary to supply irreducible minima of specified atomic groupings or complexes of nitrogen, carbon, hydrogen and so forth which, it appears, are essential constituents of living matter, and yet are not synthesizable by animal tissues. Thus the **Pyrrole** grouping, for example (see Chapter XV), which is an essential building-stone of **Hemoglobin**, would appear to be as much an elementary requirement of animals as nitrogen or carbon itself, inasmuch as, according to Abderhalden, they are unable to synthesize it from other carbon or nitrogen complexes in the diet and, lacking it, are just as assuredly suffering starvation as if they were lacking one of the more elementary desiderata.

The variety of these essential constituents of the diet with which we are acquainted is already very great and is unquestionably destined to grow with increasing scope and refinement of investigation. It is highly probable that many of the raw materials from which the various

Internal Secretions are synthesized are dietary constituents of this essential type, for example the **Iminazolyl-grouping**, which in all probability forms an essential constituent of the active principles of both lobes of the pituitary body, the **Catechol-grouping** which is an essential complement of the molecule of **Adrenalin**, and the **Indole** radical which, from the observations of Kendall, would appear to be a component of the active principle of the thyroid, are examples which will serve to illustrate the essential importance of specific molecular groupings or arrangements of atoms, which, if not synthesizable by animal tissues, must necessarily form a part of the diet in order to maintain bodily equilibrium; and to a still greater extent, of course, in order to render normal growth a possibility.

The **Vitamines**, which appear to be nitrogenous substances closely related to the **Purines**, are dietary constituents of this type. They are essential for growth, and even for the maintenance of bodily equilibrium, yet the amount required to maintain the weight of the body or to permit satisfactory growth is extremely minute. They evidently represent a group of non-synthesizable essential constituents of living matter which would appear not to be excessively complicated in structure since they are usually obtainable in crystalline form and their relationship to the pyrimidines and the purines has frequently been established.

Then, again, there are fatty constituents or substances soluble in **Fats** which are probably of a more complex character and which are equally essential elements of a complex dietary. According to the older view of metabolism, fats and carbohydrates were considered to be mutually replaceable in the dietary in isodynamic, *i. e.*, equicalorific proportions. Provided the fats in the dietary be not too greatly diminished this is still recognized to be true, but it has now been repeatedly shown that development and maintenance upon an absolutely fat-free diet is impossible, no matter what excess of carbohydrate may be furnished and, furthermore, that **Vegetable Oils** do not supply this deficiency. According to McCollum the essential constituents of the diet, in addition to the requisite mineral salts, amino-acids and calorific value in the form of fats or carbohydrates, fall into two groups, of which one is soluble in water, while the other is insoluble in water and is soluble in fats and in fat-solvents such as ether. It is immaterial from what source these constituents may be derived; provided merely that they are both present and the diet conforms to the other requirements outlined it will suffice to maintain life and permit growth. These substances have been provisionally designated by McCollum "Fat-soluble A" and "Water-soluble B"; we are as yet ignorant of their structure or affinities. But from their essentiality for growth, and even maintenance for any prolonged period, we may infer that they are **Substrates** or raw materials which are required in the manufacture of living tissue and cannot be synthesized by the tissues themselves.

The clearest indication of the dependence of tissue-synthesis upon the

presence of specific atomic groupings in the dietary is, however, afforded by the investigations of Hopkins and Willcock and of Osborne and Mendel upon the ability of various pure **Proteins** to supply the nitrogen-requirements of growth and maintenance.

We have seen that the various protein constituents of the tissues and of the diet are built up out of varying permutations and combinations of a limited number (nineteen in all) of **Amino-acid** radicals which are linked together in long chains. Now certain of these nineteen radicals are lacking in some of the proteins, and the administration of such proteins to growing animals as the sole source of nitrogen in the diet enables us to ascertain whether the amino-acid which is lacking is synthesizable by animal tissues, for synthesized it must be if normal tissues are to be produced by the animal and it is not procurable preformed in the diet.

From the investigations cited it appears very probable that the only amino-acid radical which is synthesizable by animal tissues is **Glycocoll**, or **Amino-acetic Acid**. Of the remainder, it is probable that all must be present preformed in the diet in order to permit the accretion of living tissue; at all events this has been positively established for several of the amino-acid radicals, for example **Lysine**, **Tryptophane**, **Tyrosine**, and **Cystine**.

The alcohol-soluble protein of maize, **Zein**, is lacking in glycocoll, tryptophane and lysine, and the investigations of Hopkins and Willcock and of Osborne and Mendel have shown that if Zein be the sole source of nitrogen in the diet, not only is accretion of fresh tissue impossible, but the maintenance of that already formed is also impossible, so that when supplied with abundance of nitrogen, carbon and salts in correct proportion, water and calories, the animal nevertheless dies of inanition. If tryptophane be added maintenance becomes possible, but not growth. On such a diet, or if supplied with **Gliadin** which lacks only glycocoll and lysine, a young animal lives but ceases to grow and maintains an infantile appearance, and *full capacity to grow upon readmission of the lacking constituent to the diet*, until what would normally be a "ripe old age" (Fig. 33). Upon addition of lysine as well as tryptophane, normal growth and maintenance are at once rendered possible, the glycocoll being synthesized by the animal itself. Evidently the **Endogenous Metabolism**, or waste incidental to and an essential consequence of life, of the amino-acid lysine is reducible to zero, possibly because a limited supply of lysine may be utilized over and over again in the processes of waste and repair, while, on the contrary, the endogenous metabolism of tryptophane is not reducible to zero, possibly because it is employed, not only in the manufacture of tissue, but also of constituents of the body which undergo irreversible consumption. The result is, at all events, that an inevitable waste of tryptophane attends the maintenance of life, and in its absence from the diet, the tissues of animals being unable to synthesize it from other nitrogenous constituents of the diet, tissue-waste can no longer be accurately

balanced by tissue-repair, and continuous loss of tissue on an otherwise abundant diet is the inevitable outcome.

FIG. 33.—*A* and *B* show the contrast between two rats of the same age, one of which, *B*, has been stunted by receiving a diet (protein-free milk and gliadin), deficient in lysine. The lower two pictures afford a comparison between two rats of the same weight but widely differing in age. The older, stunted rat, *B*, has not lost the characteristic proportions of the younger animal, *C*. (After Underhill.)

It has long been realized that **Gelatin** is not in itself an adequate protein for the maintenance of nitrogenous equilibrium, although it is a "sparer of protein," *i. e.*, can furnish a portion, but not the whole of the nitrogen in the diet. We now recognize that this is due to the absence of tyrosine and tryptophane from the molecule of this protein.

Casein is an inadequate protein on account of its deficient content of cystine (Fig. 34). In milk this deficiency is supplied by **Lactalbumin.**

In addition to the various dietary constituents which have been definitely ascertained to be essential and irreplaceable there are others which we can infer, from known data, to be equally essential. Thus **Cholesterol** has been shown by Gardner and his collaborators not to be synthesized by animal tissues; the cholesterol in the blood and tissues being proportionate to the cholesterol derivable from the dietary. Now cholesterol is an essential constituent of nervous tissues, and derivatives of cholesterol, such as the bile-acids, play an essential part in the bodily economy. A diet lacking in cholesterol, which is not at the same time lacking in other essential dietary constituents, is very

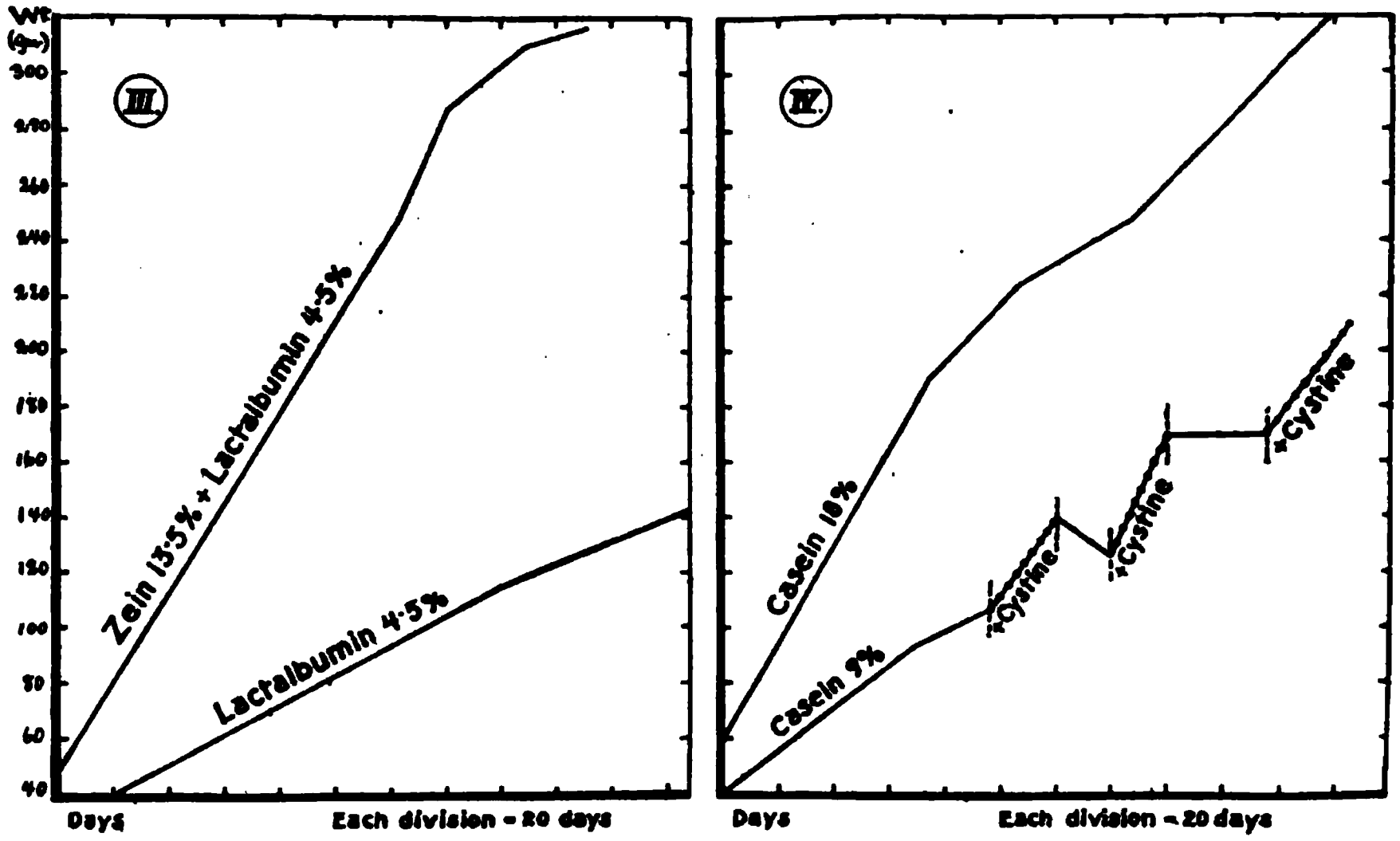


FIG. 34.—Curves of growth of rats on basal rations plus casein, showing effect of addition of cystine to an inadequate allowance of casein. (After Osborne and Mendel.)

difficult to devise, so that direct proof of its essentiality in the dietary has not yet been adduced. Still we may infer with a fair degree of confidence that a certain minimal content of cholesterol in the diet is requisite, if not for maintenance, then at least for normal growth and development.

From these various investigations it is clear that the elementary substrates of growth are in all probability very numerous and represent a variety of chemical genera, or at any rate, lipoidal substances, basic substances and amino-acids, superadded to the more elementary requirements of nitrogen, carbon and calories.

It is furthermore evident that growth, like all other chemical transformations, is absolutely dependent upon its raw materials or substrates, and cannot occur in their absence. On the other hand, the

capacity to grow, as the above-cited investigations of Osborne and Mendel reveal, is not determined by age but, as we have already concluded upon other grounds, by a lack of balance between the forward and opposed reactions of tissue-synthesis and tissue-degradation, so that upon admission of the necessary substrates, no matter what the age prior to the death or senescence of the animal may be, growth occurs and continues until equilibrium, or equality of the velocities of tissue-synthesis and tissue-degradation is attained. We thus reach once more, and from a totally different angle, the conclusion that the relatively stationary weight of an adult animal is determined by the accumulation of the **Products of Growth**, and not in any sense by the exhaustion of its **Substrates.**

THE RELATIONSHIP OF THE ENDOCRINE ORGANS TO GROWTH.

We have seen that the chemical processes which underlie the growth of animals are of such a nature that they produce their own catalyzers. But if this be so then we are immediately impelled to the conclusion that **Catalyzers of Growth** exist, *i. e.*, substances which, perhaps in minute proportion, and certainly quite independently of their nutritive or substrate- value may profoundly modify the growth of living tissues. The question now arises whether any evidence other than evidence of this inferential kind is obtainable of the veritable existence of such endogenous catalyzers of growth?

In the simpler undifferentiated organisms the catalysis of growth, in common with all the other vital processes, is doubtless a function of every cell, and each cell contains the necessary materials for the acceleration of the production of living matter. In the higher and more differentiated organisms, on the other hand, it is not at all improbable that the function of growth-catalysis is, to a greater or less extent, delegated to special cell-groups or organs, just as the function of motility is delegated to muscle-cells, that of conductivity is especially displayed by nerve-fibers, and those involved in digestion are delegated to the alimentary canal and dependent organs. We are thus led to direct our attention to the possibility of the existence in the body of special cell-groups exercising to an exceptional degree the function of growth-catalysis.

The profound significance of certain of the various **Endocrine Organs** or glands of internal secretion in the processes of growth immediately suggests that these are the special cell-groups to which the function of growth-catalysis is most particularly delegated. We know from abundant clinical experience that disorders of the thyroid, thymus, sexual glands and particularly of the anterior lobe of the pituitary body, are reflected in a profoundly disturbed development of the various tissues of the body, while the action of the secretions of the **Corpora Lutea** in stimulating the outgrowth of placentæ from the wall

of the uterus is a striking example of the intensity and specificity of growth-stimulation which may be brought about by agencies of this type.

It is possible that not all of the organs of internal secretion which are capable of affecting and modifying the growth of animals do so by virtue of growth-catalyzers which they elaborate. Thus hyperactivity of the thyroid leads to generalized loss of body-weight owing to a marked increase of metabolism and particularly of nitrogenous metabolism, while hypo-activity leads to the peculiar maladjustments of development which characterize the condition of myxedema. These effects, however, are more probably due to a general action of the thyroid principle in accelerating **Exogenous Metabolism** and reducing the nutritional level in the tissue-fluids. They are effects which more probably concern the concentration of the available substrates of growth than the specific rapidity of their elaboration into protoplasm. The disproportionate growth of connective tissues which characterizes myxedema is more probably to be attributed to the absence of the normal competition with the cellular elements for a limited supply of substrates than to any specific stimulation of connective-tissue synthesis.

The function of the **Thymus** in growth is obscure and its true significance may perhaps be rather that of a storehouse of substances, for example **Nucleic Acids**, which will be required in subsequent development than of a factory of growth-catalyzers. The relationship of the anterior lobe of the **Pituitary Body** to the processes of growth is, however, clearer and more defined, and is of such a character as to encourage the supposition that in the hypophysis we have one instance among others of an organ in which the function of growth-catalysis is concentrated and specialized.

The relationship of the pituitary gland to certain remarkable disturbances of growth was first pointed out in 1888 by the French surgeon Pierre Marie, who drew attention to two types of anomalous growth which postmortem examination showed to be invariably associated with abnormalities of the hypophysis. These rare pathological conditions are **Gigantism** and **Acromegaly**.

There are occasional individuals in whom, either before or during adolescence, the growth of the skeleton undergoes an extraordinary acceleration so that they attain such an abnormal stature as to attract universal attention. Such are the individuals who are occasionally exhibited as "giants" in shows and fairs (Fig. 35). A closer inspection of these cases usually reveals other abnormalities which, in the adult at all events may be of two opposite types. The skin may be thin, transparent and hairless, the extremities small, muscular energy deficient, the genitals imperfectly developed, and, according to Cushing, a decided intolerance for sugar is usually also present. On the other hand cases may be encountered in which the reverse of these characteristics may be noted, the skin is thick, coarse and hairy, the extremi-

FIG. 35.—Preadolescent hyperpituitarism resulting in gigantism. Height, 8 ft. 3 in.; weight, 275 pounds. (After Cushing.)

ties are more or less enlarged and the development of the sexual organs may be exaggerated. These latter symptoms are, however, more commonly displayed in the second type of anomalous development of hypophyseal origin, that afforded by the instances of **Acromegaly**. In these individuals the symptoms do not usually supervene until maturity has been attained and the epiphyses of the bones have hardened so that growth in length is no longer possible. The extremities of the bones become enlarged so that the phalanges of the fingers, for example, are

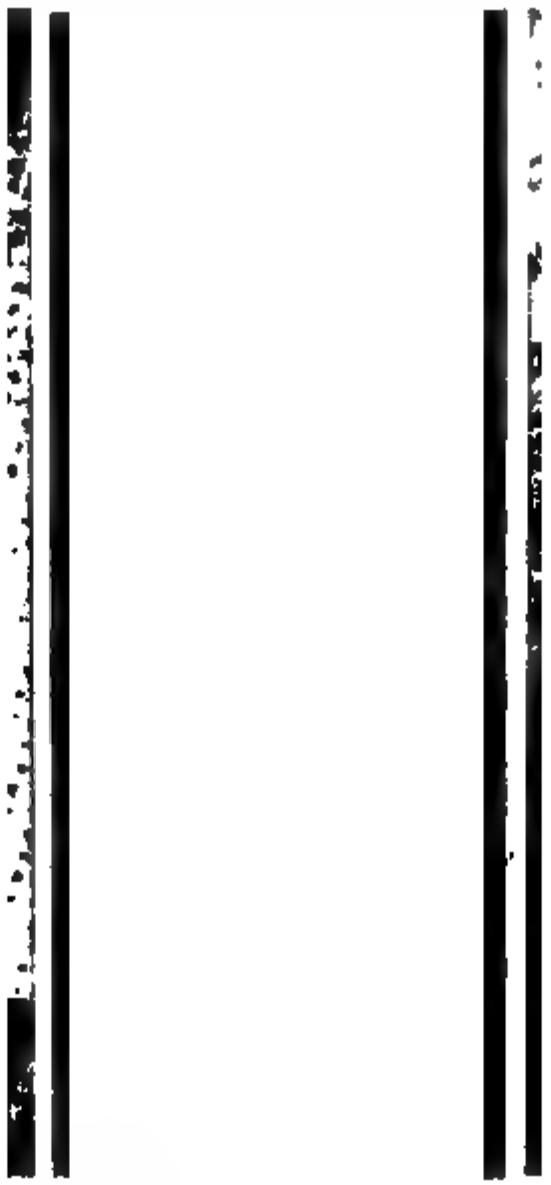


FIG. 36.—Acromegalic gigantism. Height, 6 ft. 1 in.; weight, 247 pounds. (After Cushing.)

spatula-shaped. The bodily weight becomes excessive, so that these individuals also, especially if above the average in stature, may from time to time be exhibited as giants. The features are coarsened and thickened and there is an extraordinary development of epithelial tissue and of epithelial appendages. The development of hair all over the body may be so excessive as to lend to the individual, especially when conjoined with thickened and distorted features and massive development of the jaw, a truly simian appearance (Fig. 36). Close

examination usually reveals disturbances of vision resulting in contraction of the visual field. The sugar tolerance may be abnormally high or abnormally low. Violent headaches and periods of unconsciousness or mental confusion are frequently experienced.

The ultimate fate of these cases is usually heralded by loss of muscular power and a train of symptoms which invite the supposition that the fundamental condition from which the original abnormalities arose

FIG. 37.—Dystrophia-adiposo-genitalis. Age, fifteen years; gain of 124 pounds in fourteen months. (After Cushing.)

has become reversed. Examination of the skull by means of the *x*-ray usually results, both in these cases and in the instances of gigantism, in the discovery of a decided enlargement of the *sella turcica*, or bony cavity in which the pituitary body is enclosed. Post-mortem examination usually reveals a tumor in the neighborhood of the pituitary body, either a sarcoma of the gland itself or a tumor exterior to the gland but pressing upon it.

It was pointed out by Marie that when the hypophyseal disturbance

begins before adolescence the effect is to produce gigantism, while if the disturbance supervenes after the attainment of maturity acromegaly is the result. He regarded the two conditions as differing aspects of one and the same disease, of which the symptoms were in both instances attributable to hyperactivity of the hypophysis followed ultimately by its destruction. Subsequent investigators, and especially Cushing, have confirmed this view by more extended observation, but they have also added a third type of pituitary disturbance, which is designated Fröhlich's disease, or **Dyspituitarism.** These cases may occur in childhood (Fig. 37) or in adults. They are characterised by exten-

FIG. 38.—Fat undersized animal on left has undergone partial hypophysectomy. Animal on the right is a normal animal of same sex and litter. (After Cushing.)

sive deposits of subcutaneous fat, the skin is thin, transparent, and hairless, and the sexual organs and functions are usually undeveloped. Muscular energy is at a very low level, the intelligence is usually normal but slow. These cases have in some instances been markedly alleviated by the administration of pituitary tissue or of pituitary and thyroid tissue or extracts combined, and they apparently arise from deficient activity of the hypophysis without the preliminary stimulation which is responsible for the characteristic symptoms of gigantism or acromegaly.

Experiments upon animals have shown us that while in mammals excision of the posterior lobe or *pars nervosa* of the pituitary body may be endured, complete excision of both lobes of the gland is fatal. Par-

tial excision leads to underdevelopment and particularly to retarded development of the bones (Fig. 38). In amphibians complete removal of both parts of the hypophysis is possible at a very early stage of development and Smith has shown that in hypophysectomized tadpoles development and **Metamorphosis** are very strikingly retarded in comparison with the normals, while the skin remains unpigmented and the tadpoles have the appearance of albinos. The albinism, but not the defective development, may be cured or prevented by the administration of posterior-lobe extract.

Feeding experiments in which pituitary tissue is administered to normal animals have yielded uniform, but by no means striking results. The **Posterior-lobe** tissue leads to loss of weight and intestinal disturbances which are not attributable to or indicative of any effect upon growth. The administration of **Anterior-lobe** tissue to rats has been observed by Aldrich and by Schäfer to cause retardation of early growth, followed, in Schäfer's experiments, by a secondary acceleration. Wulzen and Maxwell, working with fowls, likewise obtained retar-

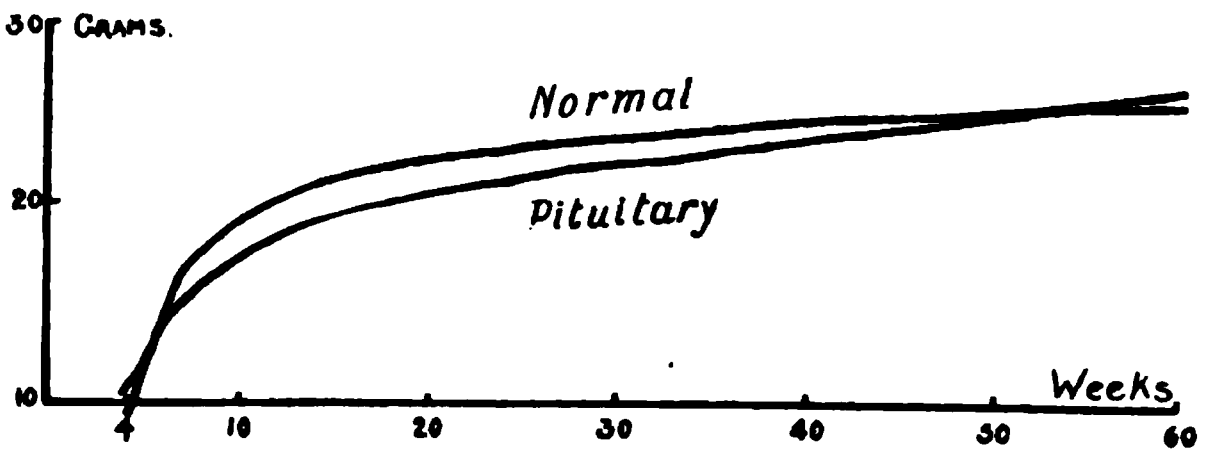


FIG. 39.—Comparison of the growth-curves of normal and of pituitary-fed female white mice.

dation followed by acceleration and the same effect has been observed in mice (Fig. 39). The uniform testimony afforded by all of these experiments is therefore that the administration of anterior-lobe tissue causes initial retardation and a secondary acceleration of growth, but both of these effects are slight.

The inconspicuous character of these results is probably to be attributed to the fact that of all the tissues of the body, the **Anterior Lobe** of the pituitary gland is the one most richly supplied with blood. The circulation is in fact extraordinarily efficient and we may infer that the active product or products of the gland leave it very rapidly and do not accumulate therein. Hence the dosage of the active material which happens to be present in the gland at the moment of death of an animal may represent but a fraction of the quantity which is manufactured and discharged in the course of a day. When we administer pituitary tissue we are seeking to imitate or accentuate by a single daily administration of merely residual material, the action of a gland which is engaged every moment of the day in manufacturing and discharging the substance which influences the growth of tissues; we cannot, therefore, look for large results. As we shall see, much more decisive effects can be elicited by the administration of a con-

centrated extract of the tissue, representing a much larger dose of the fresh tissue than would be practicable to employ. The **Posterior Lobe** of the pituitary is but poorly supplied with bloodvessels and hence the active material which it elaborates accumulates in the tissue and very minute doses of posterior-lobe tissue or extract are capable of eliciting the characteristic effects of **Pituitrin** upon smooth muscular tissue.

The **Pineal Gland** is stated by McCord to have a decisive influence upon the growth of the **Secondary Sexual Characters.** Tumors of the pineal gland have not infrequently been described, and are usually associated in children with extraordinary precocity of sexual development. Either, therefore, the pineal gland elaborates a principle which directly and specifically accelerates the growth of the secondary sexual characters, or else it operates indirectly, by stimulating the interstitial cells of the ovary or testes.

The relationship of the **Nervous Tissues** to the growth of the whole organism is one which can by no means be overlooked in this connection. It is, indeed, not at all improbable that the nervous system performs the dual role of a conducting and coördinating mechanism and a factory of endogenous catalyzers of growth. As we shall see, the growth-catalyzers of which we have positive knowledge, **Cholesterol, Lecithin** and **Tethelin,** are all lipoidal in character and these substances, or substances related to them, are exceedingly abundant in nervous tissues. We cannot suppose that the substances which contribute to the building up of nervous tissues or result from their degeneration are not abundant in the circulating fluids in proportion to the development of the nervous tissues or the ratio of their mass to that of the whole body, and several of them we know to exert, and others we may reasonably suspect of exerting, effects analogous to catalysis upon the growth of other tissues. The development of the nervous system may thus be instrumental in determining the development of the whole body.

THE METABOLIC RATE AND THE PARTITION OF NUTRIENTS.

The loss of weight which occurs in **Starvation** is by no means uniformly distributed throughout the body. The following table displays the loss of substance, in percentages of the normal weight, of the various tissues of cats after death from inanition:

Tissue or organ.	Loss of weight, per cent.
Fat	97
Spleen	67
Liver	54
Testes	40
Muscles	31
Kidneys	26
Skin	21
Intestine	18
Lungs	18
Pancreas	17
Bones	14
Heart	3
Central nervous system	3

It will be observed that those organs which are most essential to the preservation of existence are those which suffer least extensively from the unbalanced tissue-degradation which results from the fall of the **Nutrient-level** consequent upon deprivation of food. This must be due to some definite peculiarity of the metabolism of those tissues which so especially maintain their weight under these adverse circumstances. The nature of this peculiarity may be inferred from the fact that the speed of metabolism is exceptionally great in just those tissues, the **Heart** and **Nervous System**, which most successfully resist the disintegration-effects of inanition. Thus the heart is constantly transforming large amounts of potential energy into mechanical work, the maintenance of life in the higher *Metazoa* depends in fact upon its doing so, and yet it carries within itself an extraordinarily small reserve of energy-yielding materials. The **Glycogen-content** of the muscular tissues of the heart, instead of being exceptionally high, is, as a matter of fact, exceptionally low. The heart must thus depend for the maintenance of its exertions upon the direct and constant withdrawal of nutrient materials from the circulating fluids. In so doing it is forced to compete with all the other tissues of the body and yet it does so with so much success that whereas the majority of the other tissues lose a very considerable part of their weight, the heart maintains the integrity of its substance until death is imminent. This implies that the rate of utilization of nutrients by the heart must greatly exceed that of the other tissues, so that the foodstuffs are appropriated in advance of the ability of other tissues to do so.

The high **Metabolic Rate** of the central nervous system may be inferred from the fact that its consumption of oxygen is exceptionally great. The first effect of deprivation of oxygen is to arrest the higher activities of the central nervous system and those substances which paralyze the oxidizing enzymes, such as the **Cyanides**, arrest the activities of the central nervous system before any other tissue is affected to a comparable degree. The intensity of **Oxidations** in the central nervous system testifies to the rapidity of the destruction of its constituents. The fact that it maintains its integrity even in starvation, therefore, implies a proportionate rapidity of reconstruction.

The synthesis of the various tissues of the body from the foodstuffs which are contained in the circulating fluids may be regarded as a multitude of parallel reactions, all consuming similar substrates although not in identical amounts and proportions. Now in any group of **Parallel Reactions**, that is, of reactions which are occurring simultaneously and consuming the same raw materials, each substrate which enters into the reactions is shared between them in proportion to the *velocity* with which they occur. The various reactions proceed at their own independent rates and if the quantity of materials available for transformation were unlimited, each reaction, or the synthesis of each particular kind and type of tissue, would go forward at the same speed as it would if the other tissue-syntheses were not occurring simul-

taneously. The quantity of available substrates or **Nutrient-level** of the tissue-fluids is, however, not unlimited but adjusted, as we have seen, by a dynamic equilibrium, to the average needs of the body as a whole. In the competition for these materials, therefore, the most specifically rapid syntheses will have a decided advantage over the specifically slower syntheses, and when the nutrient-level sinks below the normal, as in starvation, the more rapidly metabolizing tissues will maintain their integrity for relatively prolonged periods at the expense of the more slowly metabolizing tissues.

If we now turn to the question of the origin of the varying metabolic rate of different tissues, we can only infer that the rapidly metabolizing tissues produce **Endogenous Catalyzers** of growth which are either more efficient accelerators than those which are produced by other tissues or else are produced in greater amount. We may thus clearly look to the nervous system and the tissues of the heart as the origin of very powerful or abundant catalyzers of growth. Since the majority of catalyzers, and probably the growth-catalyzers also,[1] accelerate both the forward and the backward reaction, both the anabolism and the catabolism of such tissues are exceptionally rapid.

Since the effect of starvation is to favor the rapidly metabolizing tissues at the expense of those of slower metabolic rate the result must be to increase the proportion of rapidly metabolizing tissues in an animal and the production of growth-catalyzers per kilo of body-weight. Corresponding with this fact Osborne and Mendel found that a period of starvation greatly improves the subsequent utilization of foodstuffs, so that in a growing rat the total growth attained in a period of starvation followed by a period of feeding may exceed that attained by normal animals in a like period of time. A second period of starvation even enhances this effect. The same effect may often be noted in infants as a result of a period of subnutrition or of a lowered nutritional level due to the enhanced exogenous metabolism in fevers.

From quite another avenue of experimental investigation the conclusion may also be drawn that a period of starvation increases the proportion of vigorously metabolizing tissues in the body. **Embryonic Tissues** and rapidly growing tissues generally have been shown by many observers, and particularly by Cramer, to contain a high proportion of **Water**, while those which metabolize most slowly and suffer most in any severe competition for nutrients contain a relatively low proportion of water. The nervous system, for example, contains an exceptionally high percentage of water. Now Aron has shown that a period of starvation or subnutrition leads both in children and in animals to a greater loss of nitrogen and calories than would normally be equivalent to the loss of body-weight; in other words the tissues are becoming progressively more dilute and of less calorific value.

We are led again in this connection to recall the important observa-

[1] We may infer this from the symmetry of the curve of growth.

tion of Child that starving planarians undergo retrogression to a relatively embryonic character. Child accounts for this **Rejuvenescence** by the sweeping out from the cell of accumulations of colloidal substances which impede the cell-activities and are consumed in starvation for purposes of furnishing energy. The nature of the impediment constituted by these substances is, however, by no means clear; but it may very conceivably be possible that a high proportion of water is essential to the production of growth-catalyzers in abundance. The relative rejuvenescence of metazoa by starvation is, however, more probably to be attributed to the ascendency in mass and numbers acquired by the tissues which are normally possessed of a high metabolic rate, which enables them, when food is readmitted, to push forward all of the processes of growth, including the growth of slowly metabolizing and water-poor tissues, with unusual energy.

CATALYZERS OF GROWTH.

If a catalyzer is of the "typical" variety and is not in any degree consumed during the reaction which it accelerates, then it necessarily follows that it cannot alter the final **Equilibrium** of the reaction, for a shift in chemical equilibrium means, generally speaking, that heat is either produced or absorbed and the equivalent in work or heat must be supplied by agencies external to the reaction itself, or by some other collateral chemical reaction. Since the catalyzer introduces no condition not implied in its presence, the energy-change involved in a shift of equilibrium would of necessity be equated by a change in the energy-content of the catalyzer which could only be supplied by its chemical transformation, *i. e.*, by consuming it. It follows, of course, that a catalyzer cannot *initiate* a chemical reaction which is not already proceeding, however slowly, in its absence.

If **Endogenous Catalyzers** of growth really exist, therefore, we should expect them to display the following characteristics, distinguishing them more or less clearly from the growth-substrates:

1. Since these catalyzers are not the only, nor necessarily quantitatively important constituents of the tissues which are the sum of the products of growth, it follows that the effect of catalyzers of growth may be totally disproportionate to their nutritive (*i. e.*, calorific) value.

2. The ultimate growth attained by two groups of animals under the influence of unequal amounts of the catalyzer may be expected to tend toward equality, since the ultimate station of equilibrium of a reaction is unaffected by a catalyzer, although the velocity with which equilibrium is attained may be profoundly affected. This tendency is, however, limited by three groups of factors, namely (*a*) the mechanical delay or prevention of growth which may be imposed upon an animal by the formation of a skeleton or of a circulatory or respiratory system of limited dimensions. (*b*) By the unequal effect of catalyzers upon

different types of tissue, leading, as we shall see, to the favoring of tissues of high metabolic rate, other tissues being retarded in their growth by the successful competition of the favored tissues. (*c*) By the onset of senescence, which ultimately terminates and prevents the full fruition of the growth-process.

3. Growth may take place in the absence of catalyzers added to the diet, since they are produced by the growing tissues themselves or by organs to which this particular function has wholly or partially been delegated. The growth-catalyzers are therefore not essential dietary constituents in the sense in which the growth-substrates are essential.

4. Growth-catalyzers may be expected to appreciably influence the rate of growth even when superadded to an already varied and abundant diet, whereas, in normal animals, provided all of the growth-substrates be present in the dietary in abundance the addition of a particular substrate in excess merely leads to enhanced exogenous metabolism of that foodstuff and not to enhanced utilization for tissue-building.

5. There is no reason to assume that the growth-catalyzer for any one group of tissues, is necessarily identical with that for any other. On the contrary we have evidence, as in the effect of the interstitial cells of the testes or ovaries upon the growth of secondary sexual characters, and of the secretions of the corpora lutea upon the development of the placenta, that growth-catalyzers may exist which are specific for individual tissues. Growth-substrates, on the contrary, facilitate growth as a whole, and although at a low nutrient-level the high metabolic rate of certain tissues may enable them to appropriate the lion's share of the foodstuffs, yet under normal conditions all tissues are similarly affected in differing degrees by the various growth-substrates.

6. Growth-catalyzers will be unable to initiate new growths, just as other catalyzers are unable to initiate the reactions which they accelerate.

Several substances have been discovered to influence the rate of growth of animals and of individual tissues when administered in dosages which are devoid of nutritive significance and which correspond in all of the particulars enumerated above with the anticipated properties of growth-catalyzers. Thus if **Cholesterol** be administered either by mouth or subcutaneously to animals which have been previously inoculated with pieces of **Carcinoma-tissue**, the growth of the tumor is enormously accelerated and out of all proportion to the nutritive value which the minute dosage of cholesterol which is requisite might be supposed to have, if we did not know that as a matter of fact the greater proportion of administered cholesterol is excreted unchanged. Not only is the rate of growth, of the primary tumor, as estimated by its increase of diameter, increased by one or two hundred per cent., but the growth of **Metastases** or offshoots of the tumor in distant organs and the percentage of animals displaying metastases are very

remarkably increased. Sweet, Corson-White and Saxon had a strain of carcinoma which had never been known in their experience to yield metastases in rats. They administered cholesterol by mouth to a large number of rats inoculated with this tumor and obtained metastases in over ninety per cent. of the animals. It has also been shown by Browder that cholesterol has a remarkable influence upon the rate of multiplication of the infusorian *Paramecium*, increasing the number of generations produced in a given period by several hundred per cent.

If cholesterol be administered to young mice in dosages of 40 mgm. per day, however, a result is obtained which is at first sight rather surprising, for the growth of the animals, instead of being accelerated, is very markedly retarded during the early weeks of the third growth-cycle (fifth to fifteenth week) and subsequently undergoes a secondary acceleration which, however, never makes up for the ground lost during

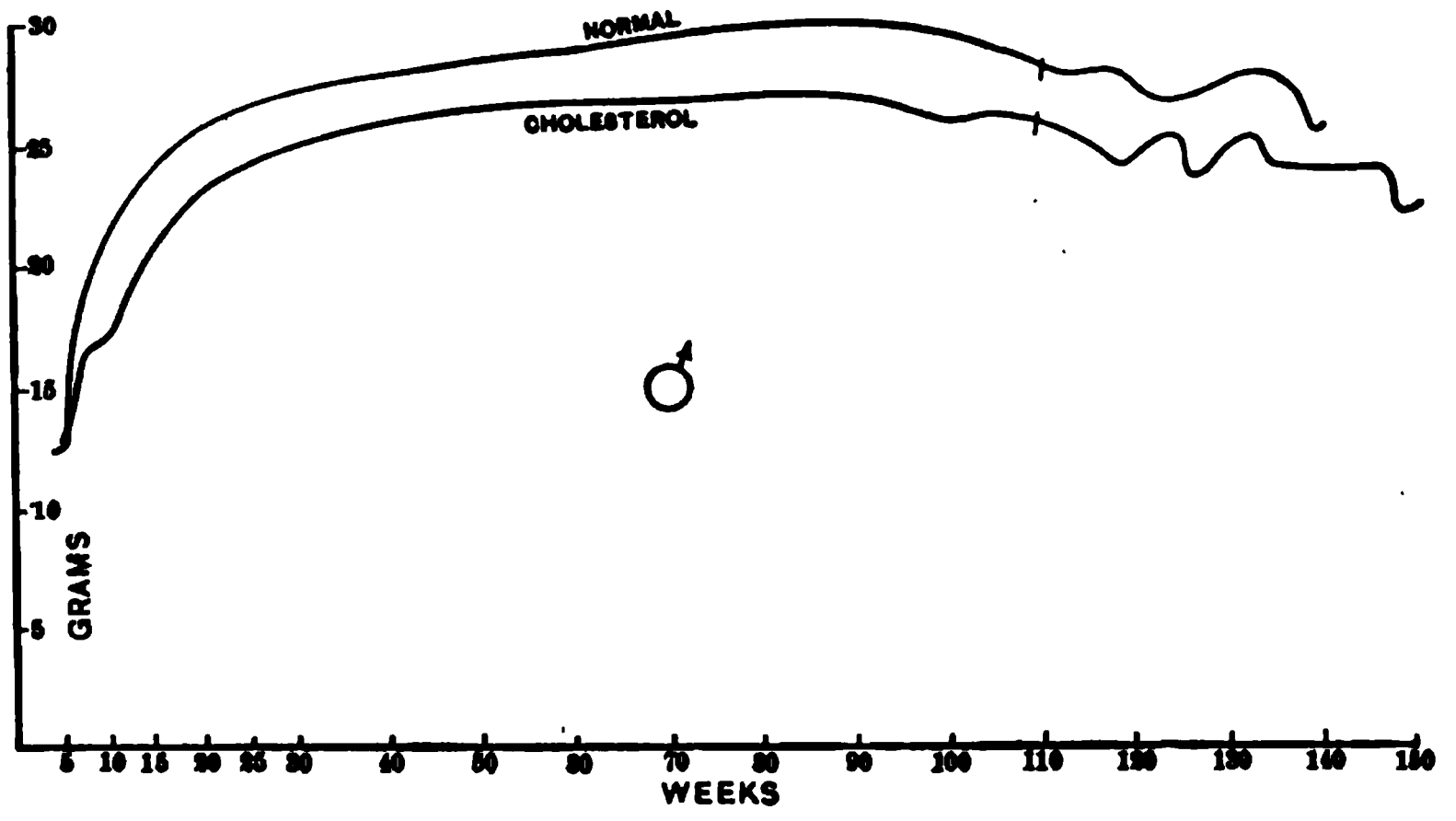


FIG. 40.—Influence of cholesterol upon the growth of male white mice. Dosage, 40 mgms. per day. The vertical cross-mark indicates average duration of life.

the period of initial retardation (Fig. 40). Now when cholesterol is administered in unusual amounts to animals the excretory mechanisms prove insufficient and large deposits are formed in a variety of organs, particularly the liver, spleen and suprarenal capsules, and it might be imagined that this or some other deleterious effect of cholesterol, superadded to its effect upon growth is responsible for the retardation of the growth in weight of animals to which its administration leads. This, however, is not the case, for this effect of cholesterol is merely a particular instance of the general action of growth-catalyzers upon the adolescent growth of animals.

It will be recollected that the administration of the tissue of the **Anterior Lobe of the Pituitary Body** to growing animals produces a like unexpected result, namely a retardation of the early adolescent growth followed by a secondary acceleration. Now hypophyseal tissue, when

emulsified and administered by hypodermic injection, brings about an acceleration of the growth of inoculated carcinoma in rats which is just as marked as that which is caused by cholesterol. By extraction with alcohol and subsequent precipitation with ether a substance is obtained from the dried tissue of the anterior lobe of the pituitary body which has been designated **Tethelin.** This substance is evidently a lipoid, for it yields fatty acids on hydrolysis, but it is a lipoid of very exceptional physical and chemical characteristics. It is soluble in water, alcohol or ether, but insoluble in a mixture of certain definite proportions of alcohol and ether. It is present in ox-glands to the extent of about 0.7 per cent. of the fresh anterior-lobe tissue. The administration of four milligrams of this substance per day to mice from five weeks of age onward produces a most decisive change in the velocity and time-relations of growth. The effect is similar in kind to that of

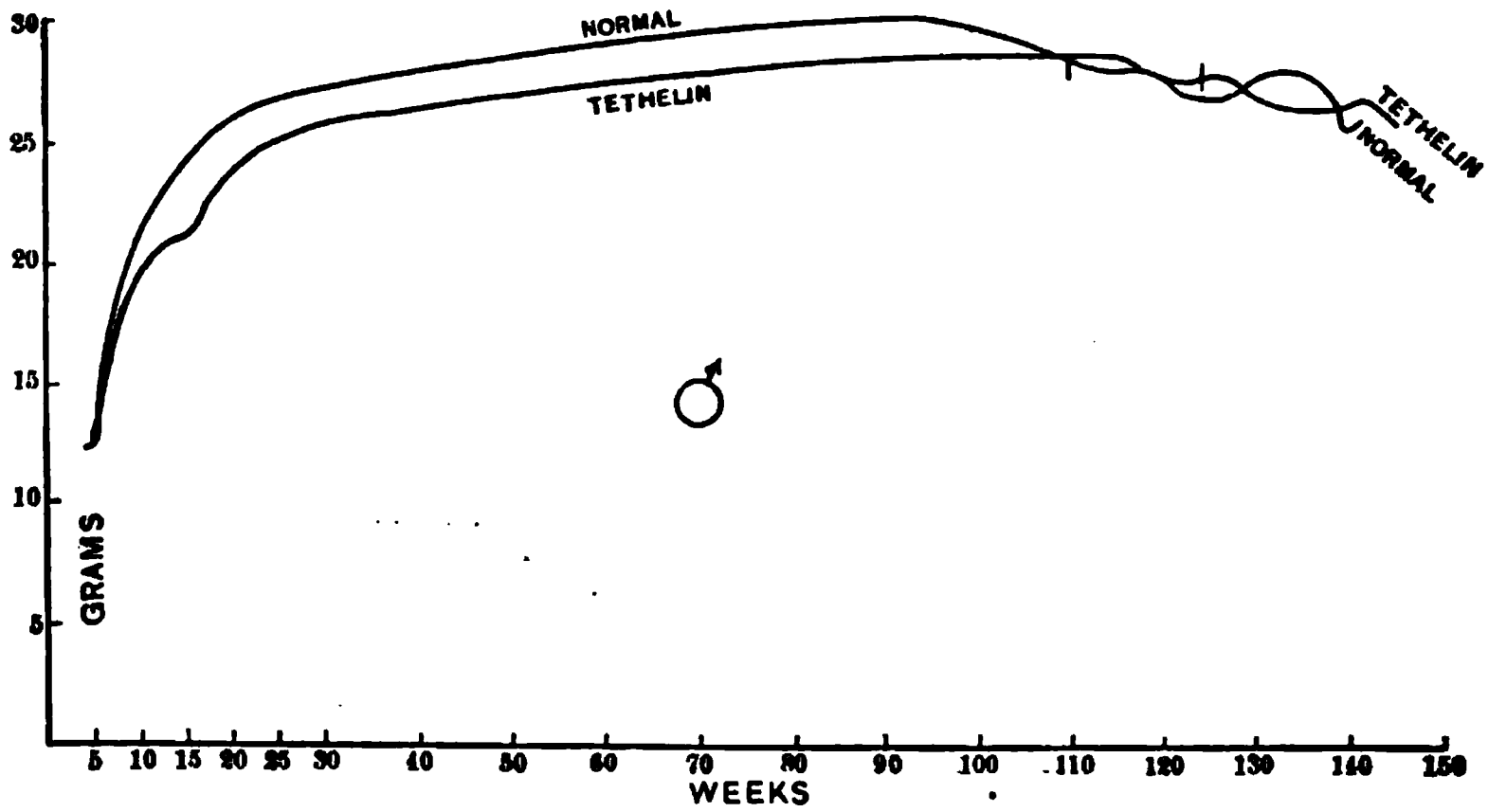


FIG. 41.—Influence of tethelin upon the growth of male white mice. The vertical cross-mark indicates average duration of life.

the administration of pituitary tissue itself, that is, initial retardation followed by acceleration, but both effects are exaggerated so greatly as to involve total distortion of the curve of growth, the second growth-cycle appearing to be prolonged while the third or adolescent cycle is abbreviated and accelerated (Fig. 41). The quantitative difference between the growth-effects obtained with tethelin and observed in anterior-lobe tissue administration are attributable to the difference in the dosage of tethelin which is received in the two cases. It is not practicable, for example, to administer much more than a twelfth of a fresh ox-gland per day to mice, because the quantity of meat consumed would otherwise constitute an important abnormality in the diet. This amount of pituitary tissue, however, contains only between eight and nine-tenths of a milligram of tethelin, or one-fifth the amount administered in the experiments cited above.

The influence of tethelin upon the growth of mice is therefore similar to the effect of administering cholesterol, save that results are attained by administration of tethelin with a tenth of the dosage that would be requisite in the case of cholesterol. It is very significant, therefore, that the action of tethelin upon inoculated **Carcinoma** in rats again reproduces the effects of cholesterol (Fig. 42.)

Even more striking than its effect upon the growth in weight of the animals is, however, the effect of tethelin upon the general contour and appearance of mice to which it has been administered continuously. The tethelin-fed animals are remarkably robust and compact in build. Weight for weight they are smaller and size for size much heavier than normal animals. The contours of their surface are more rounded and fully adult animals retain a youthful appearance which is soon lost in

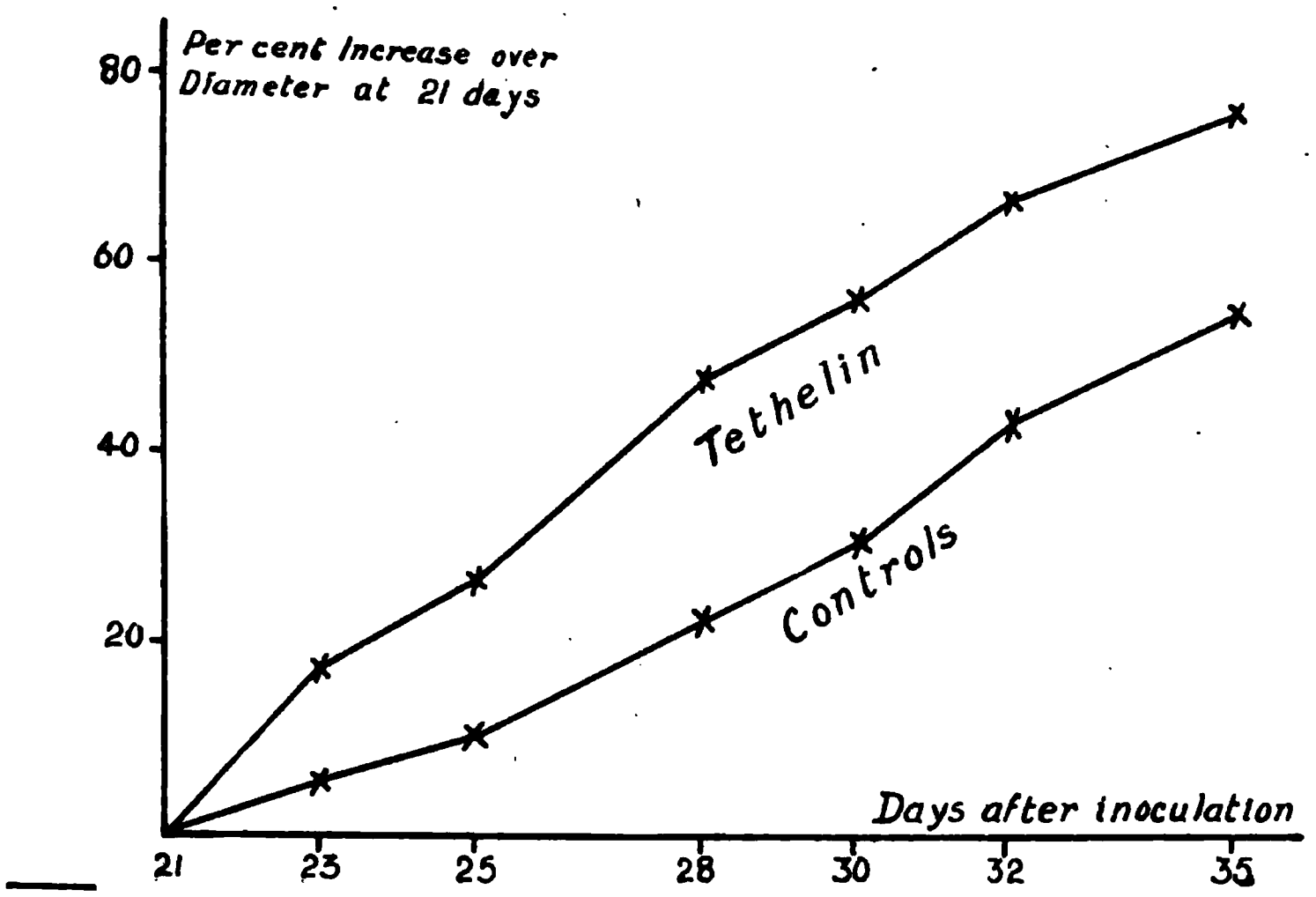


FIG. 42.—The acceleration of the growth of carcinomata (in rats) by hypodermic administrations of tethelin.

normal animals. The coats of the males, even at fourteen months of age, retain the glossy, silky appearance of the coats of young animals or of females, while six months or more prior to this age the coats of normal males are already shaggy, staring, and discolored. These differences are clearly displayed in the accompanying photograph, in which a normal and a tethelin-fed male of the same age (one year) and of the same weight (28.0 gm.) are compared (Fig. 43). The normal animal on the left has a shaggy, staring and discolored coat, while the tethelin-fed animal has a smooth, glossy and pure white coat. The normal animal is irregular in outline and loosely built, while the contour of the tethelin-fed animal is rounded and its build is compact.

In each of these three instances of growth-catalysis, therefore, we meet with the apparently contradictory fact that while the growth of a

neoplasm (carcinoma) is accelerated by the catalyzer, the growth of young animals prior to sexual maturity is retarded. It might be imagined that this constituted evidence of a fundamental difference between the metabolism of malignant tissue and that of normal tissue. This inference would not be justified, however, because in the first place no other evidence of a fundamental difference between the growth of malignant and of normal tissues has ever been advanced and, in the second place, the accelerative action of these catalyzers upon growth is not by any means confined to the growth of malignant tissues. Thus cholesterol accelerates, as we have seen, the division-rate in

FIG. 43.—Comparison of a normal (left) and a tethelin-fed (right) male white mouse, both one year old and 28 grams in weight. Note the smooth coat and compact form of the tethelin-fed mouse as contrasted with the loose form and rough coat of the normal animal.

Paramecia. Our clinical experience abundantly confirms the fact that hyperactivity of the pituitary body leads to abnormally rapid development of bony and **Epithelial Tissues** and, finally, tethelin markedly accelerates the regeneration of epithelium lost by injury and the regain of weight lost during a period of inanition after the readmission of food. The action of tethelin in hastening the repair of epithelial lesions is so decided that it has been proposed as a means of accelerating the repair of slowly-healing wounds, such as the leg-ulcers which may result from varicose veins.

We have the apparently opposed facts, therefore, that cholesterol and tethelin definitely accelerate the growth of certain types of tissue,

while the growth of the entire animal is retarded. Evidently, therefore, there are in the body certain other and relatively bulky tissues of which the growth is directly or indirectly retarded by tethelin.

The most probable reason for this retardation lies in the varying **Metabolic Rates** of the different tissues of the body and their consequent differing success in the competition for nutrients. There are, broadly speaking, two easily distinguishable groups of tissues in the animal body which differ fundamentally in function and metabolism. These are on the one hand the **Parenchymatous Tissues**, which are essentially cellular, self-maintaining cells derived from the ectoderm and entoderm of the three embryonic layers and on the other hand a variety of tissues which originate mainly but not exclusively from the mesoderm and constitute the **Sclerenchyma** or tissues of primarily structural or architectural significance. These latter tissues are dependent. They can only arise through the activities of nucleated living cells, of which they constitute outgrowths, secretions, or products of retrogressive change. Of this character, for example, are the various fibrous tissues, the elastic and calcified tissues, and the ligaments, tendons and other structures which bind together and support the tissues of more varied and complex function. The sclerous tissues have a low **Metabolic Rate**, are among those which lose most heavily in the competition for a subnormal supply of nutrients and, since they are as a rule devoid of the power of multiplication or even of repair without the intervention and assistance of other cells, we may legitimately infer that they do not produce, as the parenchymatous tissues do, **Endogenous Catalyzers** which accelerate their synthesis and degradation. In fact since their synthesis is accomplished by other cells there would be no particular purpose served by their doing so. Thus the horny cells of superficial epidermis, which have lost the power of reproduction and growth in the course of the degenerative changes which have resulted in their transformation into **Keratin**, are renewed from time to time by the multiplication of the cells of the Malpighian layer of the deeper epidermis. Cartilage and bone are similarly formed from cellular tissues and the fibrous tissues are excretions or transformation-products of the **Fibroblasts** from which they originate. Even the muscular tissues may in like manner originate from special cells which have retained the potentiality of reproduction. But if these tissues do not produce endogenous catalyzers and in many cases cannot form the material of which they are composed, it is evident that growth-catalyzers from other sources can only affect their development in the indirect fashion of promoting the growth or multiplication of the cells or other tissues from which they arise.

A catalyzer of growth may accelerate the formation of parenchymatous tissues, but its exceptional abundance or potency may actually retard the growth of the tissues which are not directly affected by it, through the deflection of nutrients to the parenchymatous elements. An important proportion of the total increment in weight of an animal

during the adolescent growth-cycle is the formation of **Connective Tissues**[1] and if the development of certain of these be retarded in the manner indicated, it may readily be understood how the rate of growth of the animal as a whole, estimated by its weight, is retarded although the growth of its parenchymatous tissues may be considerably accelerated. That this is probably the correct interpretation of the facts is furthermore shown by the effect of discontinuing the administration of tethelin to mice after the initial retardation of growth has become well marked. The secondary acceleration of growth which succeeds the retardation in animals which received tethelin, cholesterol or pituitary tissue throughout their lives is, in this event very much enhanced, so that the effect of the initial retardation of growth is not only fully

FIG. 44.—Showing the effect of a brief period (five weeks) of administration of tethelin upon the subsequent growth of mice. Animal on the left (31 grams) is the average tethelin-treated animal at five hundred days. On the right (25 grams) an average normal animal of same age.

compensated, but a supernormal accretion of weight occurs, carrying the animals far beyond the average of normal animals of the same age. This is strikingly shown in the preceding photograph (Fig. 44), in which a female mouse of average normal weight at five hundred days of age (=25 grams) is compared with a female representing the average weight (=33 grams) of animals which had received four milligrams of **Tethelin** daily from the fifth to the thirteenth week of age; the administrations being then discontinued. The remarkable overgrowth which is thus attained is evident even in the average animal displayed in the photo-

[1] Thus Bischoff (Voit's Handbuch der Physiologie, Bd. 6, p. 511) finds that the muscular, skeletal and fatty tissues comprise 76 per cent. of the weight of the adult and only 53 per cent. of the weight of the newborn. Rubner estimates that a man weighing 60 kilos contains 37.8 kilos of cell mass of which 40 per cent. is muscular tissue.

graph, but one-eighth of the animals so treated actually attained weights in excess of forty grams, a weight which, it may be stated, no normal female mouse ever attains. This remarkable overgrowth is probably attributable to the preceding development of parenchymatous tissues. The removal of the stimulus which enabled them to predominate in the struggle for nutrients gives the sclerous tissues the opportunity to develop, and the reattainment of normal proportionality between the sclerenchyma and parenchyma finally enables the stimulation of growth which has actually occurred to find expression in the supernormal weight of the animal as a whole. The occurrence of **Acromegaly** in man may actually indicate therefore, not a present hyperactivity of the hypophysis, but a *preceding* hyperactivity, succeeded, before the onset of the acromegalic symptoms, by a normal or even subnormal activity of the gland.

It is a noteworthy fact that although the administration of **Cholesterol** or **Tethelin** to normal animals which have been inoculated with **Carcinoma** leads to acceleration of the growth of the neoplasm, yet it has so far proved impossible, despite many trials, to induce the spontaneous development of tumors in animals by the administration of these substances. The percentage of mice which develop carcinoma is the same in animals which have received cholesterol or tethelin for the greater part of their lives as it is in normal animals. In other words these substances, like the catalyzers with which we are familiar in other chemical transformations, are unable to initiate the reaction which they accelerate.[1] Moreover the spontaneous development of carcinoma is even greatly delayed and the growth of the neoplasm when it has arisen is very much slowed by the continuous administration of tethelin to animals. It would appear that the continuous administration of tethelin results in such a disproportionate development of parenchymatous tissues that they are enabled to compete successfully with the neoplasm for the nutrients in the tissue-fluids, whereas in the normal animal the neoplasm shares with the limited proportion of parenchyma the advantages of enhanced catalysis of the growth-processes.

Carcinoma is essentially a disease of old age and the investigations of Wacker have shown that the cholesterol-content of the subcutaneous fats is exceptionally high in elderly people and in persons afflicted with carcinoma. Luden has also found that cholesterol is exceptionally abundant in the blood of individuals suffering from carcinoma, while the oxidation-products of cholesterol which yield Lifschütz's reaction without preliminary treatment with oxidizing-agents, which are abundant in normal blood, are absent or scanty in the blood of carcinomatous individuals.

[1] Erdmann has described an innoculable tumor which was produced by the inoculation of foreign non-malignant tissue followed by an induced inflammatory reaction and administration of tethelin, but tethelin alone was ineffective.

OLD AGE AND SENESCENCE.

The leading characteristic of old age is the low average **Metabolic Rate** of the tissues. From maturity to old age the calorific output steadily diminishes, the total reduction, according to Du Bois, being about thirteen per cent. by eighty years of age in men. This diminished metabolism, if it is not accompanied by a corresponding diminution of intake, may lead to the formation of extensive deposits of fat and the **Obesity** which occurs in a certain percentage of elderly individuals. In general, however, the decreased metabolic rate is accompanied by a progressive loss of body-weight. In man the senescent loss of body-weight begins relatively early, but proceeds very slowly, so that it only becomes notable at an age in excess of the mean duration

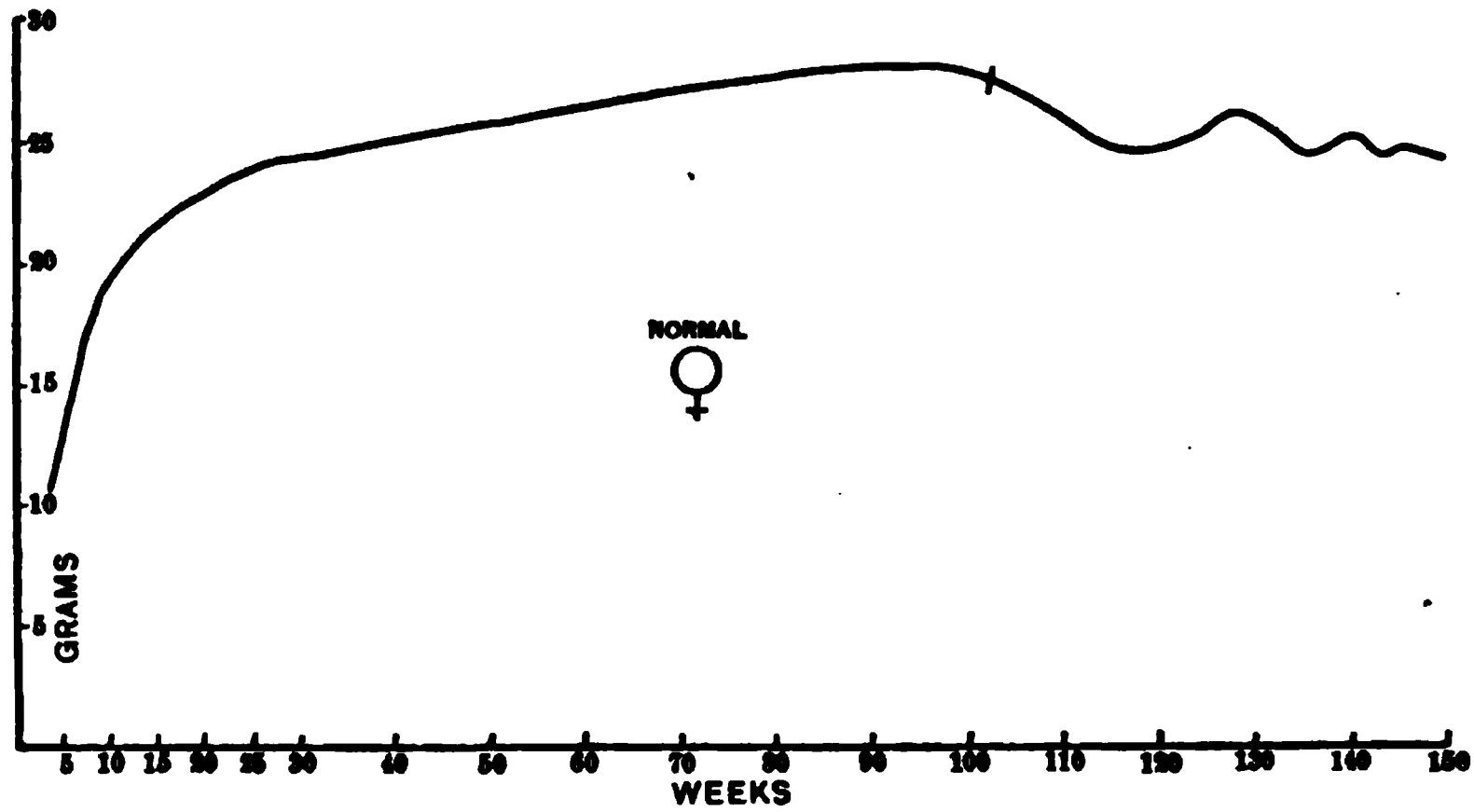


FIG. 45.—Growth curve of normal female white mice from four weeks until death of the last surviving animal. The vertical cross-mark indicates average duration of life.

of life. In the mouse, on the contrary, the **Senescent Loss of Weight** is relatively sudden and rapid and is quite marked before the mean duration of life is attained. This is illustrated by the accompanying curve (Fig. 45), which displays the growth and senescence of female white mice from four weeks until the termination of the observations by the death of the last surviving animals. Deaths from epidemic infection were excluded by the technique of the experiments. The terminal fluctuations of the curve are due to the irregularly occurring deaths of animals in which the process of senescence has been most rapid and which have lost most weight. The survivors therefore represent an earlier or less complete stage of senescence than those which have died, and each group of late deaths is consequently accompanied by a rise in the weight-curve of the survivors. Each rise, however, is succeeded by a fall, which is even more rapid than the preceding one, indicating

that the process of senescence is in reality continuous, and, moreover, that it proceeds with a regularly increasing velocity which depends upon the age rather than upon the weight of the animals. The same characteristics are displayed by the curves in Fig. 41 on p. 506.

There is no particular reason, implied in the nature of an autocatalytic process, why the mass of its product should diminish. In fact, the station of **Equilibrium** in a purely autocatalytic process, uncomplicated by side reaction, is asymptotically approached and never actually attained, so that the total mass of product, so far from decreasing at the apparent close of the reactions, is actuallly increasing at an infinitesimal rate. The process of growth, however, although it is autocatalyzed, does not conform to this particular characteristic of autocatalytic reactions and, a maximum yield of product having been attained, the tissues slowly disintegrate, even gathering speed as time proceeds, until, if no other factor intrudes to terminate life, **Senile Atrophy** of the tissues leads to irreparable weakening of some essential organ.

A variety of hypotheses have been advanced to account for the phenomena of senescence which, even if all other dangers of life could be surmounted, would set an inevitable term to existence. A very natural supposition is that proposed by Bütschli, that death is due to the exhaustion of a certain substance—the "life ferment"—which is gradually used up during life. We cannot disassociate senescent atrophy from senescent death, however, since the death of aged individuals is obviously determined by the progressive atrophy or degeneration of essential tissues. Now senescent atrophy is attributable to the inability of the tissues to maintain their weight and we must therefore, in the terms of Bütschli's hypothesis, suppose that the gradual consumption of an essential substance which was originally contained in the germ-cells and can be manufactured only by them, has deprived the tissues of the power to form new protoplasm. Now this is not the case, for even in old age, injury, or removal of the **Products of Growth**, will institute vigorous **Regeneration** and repair. The capacity to grow is not lost or even impaired by age. Thus Osborne and Mendel have maintained rats in an infantile stage of development by depriving them of the single amino-acid **Lysine**. But upon readmission of lysine to the diet, even at an age exceeding the average normal duration of life (700 days), growth is immediately inaugurated, at the same speed that it would, in the normal course of events, have taken place in a normally fed animal of similar weight and stage of development. The retardation of growth by the accumulation of the products of growth is therefore one of the important factors in determining the inability of the adult tissues to maintain their weight in aged animals. It is not the only factor, however, because in that case, as we have seen, indefinitely prolonged equilibrium and not decline would be the resultant.

A modification of Bütschli's hypothesis is that proposed by Rubner, namely, that the protoplasm of an animal is able to sustain a limited number of molecular transformations and no more. Thus he points

out that the total calorific output of a variety of animals from birth to old age is approximately the same, a striking exception, being, however, afforded by man:

TOTAL CONSUMPTION OF CALORIES PER KILOGRAM OF BODY-WEIGHT.

Man	725,770
Horse	169,900
Cow	141,090
Dog	163,900
Cat	223,800
Guinea-pig	265,500

The instances are, however, not very numerous and if one marked exception to the "rule" occurs among such a small number of cases, other exceptions will doubtless be encountered. Indeed we may with more probability attribute the exceptional position of man in this small group to the much larger proportion of **Nervous Tissues**; tissues, that is, of high metabolic rate, which his body contains in comparison with the other animals enumerated. His duration of life is also, and possibly for the same reason, exceptionally great.

Quite a different type of hypothesis to the foregoing is that proposed by Metchnikoff, who attributes senescence in part to the aberrant activities of **Phacocytes** and in part to the absorption of toxic substances which are products of bacterial decomposition in the lower intestine. While there can be little doubt that some of the tissue-changes which are characteristic of old age, such as sclerosis, vascular lesions and so forth may be hastened or even brought about by repeated administrations of basic substances, such as **Adrenaline** or **Tyramine** which may be derived from amino-acids by **Decarboxylation**, yet as a general hypothesis of senescence this is too specific, too limited in its scope and applicability, to account for the phenomenon in the multitude of the forms of life which exhibit it. In fact, Metchnikoff did not advance his hypothesis as an explanation of "natural" old age, although he is commonly accredited with having done so, but as an explanation of what he considered to be the "premature" senescence of human beings, and, as such, it is a hypothesis which deserves very serious consideration. The effects produced by basic **Nitrogenous Poisons** related to the amino-acids are, however, confined to certain tissues and especially the circulatory and renal systems, while the effects of senescent atrophy modify in greater or less degree every tissue in the body. Organisms in which the structural changes producible by poisons of this character could not constitute an irreparable injury nevertheless display senescence and its necessary outcome, "natural death."

The unicellular animals and certain unorganized types of living tissue, such as cancer-tissue, are, as Wiessmann and Loeb have especially emphasized, actually or potentially immortal.[1] The **Unicellular**

[1] Those forms which undergo periodical conjugation may also exhibit senescence, which, however, may very possibly be due to causes analogous to those described below which lead to senescence in the metazoa. Cf., G. N. Calkins: Proc. Soc. Exper. Biol. and Med., 1919, 16, p. 57.

Organisms subdivide, and the daughter-cells which thus arise each contain the protoplasm of the parent-cell which is thus perpetuated indefinitely. No slackening of the process of reproduction occurs unless the supply of nutrients fails. Even in those forms such as the *Infusoria*, in which conjugation of two cells occasionally occurs, this is not generally essential to the maintenance of the indefinite reproducibility of the original protoplasm. In the growth of **Cancer** only the failure of the tissues of the host to support the parasitic tissue sets a term to its existence. If the tissue be transplanted from time to time into a fresh host it is propagated indefinitely. The failure of nutrients is again the only factor which limits indefinite reproduction.

The mortality of higher organisms is therefore a consequence of their complexity, and a very probable explanation lies in the sub-division and delegation of functions and powers which renders this complexity possible. There is a very noticeable alteration in the relative proportions of the different types of tissue in the body with advancing age. As Metchnikoff has expressed it: "Old age is characterized by a conflict between the finer and more complicated elements and the simple or more primitive elements of the organisms, a conflict that ends to the advantage of the latter. The picture is always the same—atrophy of the more highly differentiated elements and their replacement by an overgrowth of connective tissue." In other words **Sclerous Tissues** acquire a dominance over the **Parenchymatous Tissues** which are the most important or perhaps exclusive source of the endogenous catalyzers of growth.

The senescent decay of the body may, in fact, be attributable to the increasing mass of dependent tissues with which nutrients must be shared and for the production and repair of which catalyzers must be provided. So long as the velocity of the forward reaction of growth predominates sufficiently over that of the backward reaction, the impulse to growth secures the continued accretion of tissue. Part of this tissue assists in the production of catalyzers, but part, that part constituted by the tissues of structural rather than functional significance, merely draws away nutrients from the tissues which produce the endogenous catalyzers. This has the effect, so far as the self-maintaining tissues are concerned, of progressive reduction of the **Nutrient-level**, or diminution of the value of "a" in the autocatalytic equation. The value of "a," however, determines the ultimate or equilibrium-weight of the animal and as it sinks so must the weight of the animal diminish, the parenchymatous tissues being directly and the sclerous tissues only indirectly affected. Hence the proportion of sclerous to parenchymatous tissues is further enhanced and the process of senescence itself partakes of the autocatalytic character.

It should be especially noted in this connection that the cost of production of **Sclerous Tissues** is not to be estimated merely in terms of their mass. They are "expensive" tissues to manufacture in comparison with the parenchymatous tissues. Not only are they poorer in

water and therefore richer in organic materials than the parenchymatous tissues, but the **Proteins** which they contain are of very abnormal composition, a composition which is specific for each type of sclerous tissue. They are incomplete proteins, containing certain amino-acid radicals in exceptional abundance, while others which usually occur in proteins of cellular origin are lacking or present in unusually small amounts. To manufacture one molecule of a protein of this abnormal character several molecules of the ordinary types of protein must be sacrificed, just as several buildings constructed of wood, stone and brick must be sacrificed to obtain the materials wherewith to construct a similar building entirely of stone or of brick. Hence the drain upon the nutrient-level in the circulating fluids which is brought about by the sclerous tissues is far more than proportionate to their mass.

We have seen that the administration of **Growth-catalyzers** must favor the development of parenchymatous as opposed to sclerous tissues. Corresponding with this view and with the views expressed above concerning the origin of senescence, we find that the continuous administration of **Tethelin** to mice, from the fifth week of age onward, or even its intermittent administration for several brief periods, leads to a remarkable prolongation of the average **Duration of Life**. Thus the duration of life of normal white mice was found in the particular stock employed to be 767 days for males and 719 days for females within a probable error of somewhat less than one month. Males which had received 4 mgm. of tethelin daily throughout their lives attained an average age of 866 days before death, while females intermittently receiving the same dosage attained an average age of 800 days. This would be equivalent to a prolongation of from ten to fifteen years in the average duration of life in man. Pituitary (anterior lobe) tissue, cholesterol, and lecithin alike failed to influence the duration of life, the pituitary tissue on account no doubt of the smallness of the dosage of tethelin contained in the amount of the tissue which it was practicable to administer, and cholesterol on account of the secondary deleterious effects of the deposits of this substance which accumulate in the tissues of animals receiving excessive amounts. The absence of any effect of the administrations upon the life-duration of these various groups of animals rendered them additional "controls" by reference to which the prolongation of life attained by the administration of tethelin could be gauged. The average duration of life of the tethelin-fed males was found to exceed the average life-duration of the males of all other classes of animals investigated by one hundred and three days, while the life-duration of the tethelin-fed females exceeded that of all other classes by one hundred and eight days. The chance of both of these deviations from normality being "accidental" was computed to be only 1 in 11,000. The prolongation of life in mice by the continuous or frequent administration of relatively large doses of tethelin is therefore unmistakable. Furthermore, **Senescence** is very

much delayed in tethelin-fed animals, the loss of weight for a prolonged period being almost imperceptibly gradual, whereas in normal animals it is relatively sudden (Fig. 41, p. 506).

We have seen that the tissues of the **Nervous System** are very rich in lipoids which are either identical with (cholesterol) or related to (phospholipins, etc.), the substances which we know to have an influence upon growth similar to that which we would expect to be exerted by catalyzers of growth. Furthermore their exceptionally high **Metabolic Rate** encourages the supposition that they produce an abundance of endogenous growth-catalyzers. A predominant development of nervous tissues should therefore be equivalent in its effects upon metabolism, growth, and life-duration to the continuous administration of an excess of growth-catalyzers.

Now Friedenthal has pointed out that the ratio of brain-weight to body-weight or to the two-thirds power of the body-weight, which he terms the "cephalization-factor," varies from one species of animal or bird to another in extremely close correspondence with the maximal attainable duration of life. The following are among the figures which he cites in support of this thesis:[1]

MAMMALS.

Species.	Cephalization-factor.	Maximal life-duration (according to Hanseman in years.
Man	2.67 to 2.81	80 to 150
Elephant	1.24 to 1.34	90 to 100
Anthropoid apes	0.76 to 0.65	....
Horse	0.43 to 0.57	45
Deer	0.40 to 0.50	30
Bears	0.36 to 0.50	50
Dogs	0.34 to 0.51	15 to 20
Cats	0.29 to 0.34	20
Oxen, Giraffes, Antelopes	0.30 to 0.40	30
Squirrels	0.16 to 0.20	6
Insectivora	0.06 to 0.18	6 to 10
Mice	0.04	3

BIRDS.

Species.	Cephalization-factor.	Maximal life-duration (according to Hansemann in years.
Carrion crow	0.168	100 (?)
Parrots	0.147 to 0.177	100 (?)
Alpine crow	0.114	50
Buzzard	0.11	....
Owl	0.113	....
Finch	0.086	8
Sparrow	0.086	....
Duck	0.0731	....
Snipe	0.0585	....
Quail	0.0495	....
Heron	0.0459	15
Pheasant	0.0343	15
Fowls	0.0249	10 to 20
Ostrich	0.0195	....

[1] The life-duration of the mouse computed from the observations cited above has been added to the table.

The various estimates of the maximal **Duration of Life** can only be regarded, excepting in the case of the mouse, as very hazardous approximations, since, even in the case of man, the maximal attainable duration of life has been the subject of far more fables than investigations. Probably the *mean* duration of life would be a better standard of comparison than the *maximal* duration of life, since the magnitude of the latter estimate may be so greatly affected by a single exceptional observation. On the other hand statistical estimates of the average duration of life are lacking, save for man and mice, and even the estimates for man which are available include accidental deaths and deaths from epidemic infections. However, notwithstanding the approximate character of the estimates, they afford very striking evidence of a tendency of **Longevity** to be associated with a high degree of development of the nervous system. Thus, so far as the effect upon the duration of life is concerned, exceptional development of the nervous system exerts an effect similar to that which is induced by the administration of an excess of a growth-catalyzer.

The resemblance between the effects of a high proportion of **Nervous Tissues** and those induced by administration of a growth-catalyzer extends, however, even to the time-relations of growth, as expressed by the contours of the growth-curve. Thus on comparing the growth-curves for man and mice in Fig. 31 (p. 473), with the growth-curves for cholesterol-fed and tethelin-fed mice in Figs. 40 and 41 (pp. 505 and 506), it is at once apparent that the change in the time relations of the growth of mice which is induced by these catalyzers brings their growth-curve into close approximation to the human curve. The effect of the growth-catalyzers in unusual amount is to apparently prolong the second and abbreviate and accelerate the third growth-cycle, and it is in precisely these characteristics that the human growth-curve, when reduced to the same scale, differs most strikingly from the growth-curve for mice. It is not unlikely, therefore, that the difference in contour of the mouse and human curves of growth is attributable to the greater abundance of endogenous catalyzers of growth in the tissues and tissue-fluids of man consequent upon the greater proportionate development of his nervous system.

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CHAPTER XXI.

PROCESSES INFERRED FROM INDIRECT OBSERVATION: MEMORY AND SLEEP.

MEMORY.

THE most prominent characteristic of the **Nervous System** is the facilitation of its functions which their performance brings about. A mental task which is at first difficult becomes easy by frequent repetition; an act which may be performed under the guidance of the central nervous system at first only with effort and concentration of the will and attention, becomes by repetition a habit or even a reflex which is performed almost automatically and without any conscious expenditure of effort.

Secondary and subsequent to this phenomenon of facilitation is the phenomenon of **Fatigue**. For example, in the learning of a long passage by rote, as one tries to recall it after the first repetition, recollection is distinctly difficult. With a second repetition recollection is easier, with a third it is easier still and so the progressive facilitation accumulates until it becomes possible to repeat a long passage from "**Memory**," faultlessly and fluently. If, however, the repetitions be still continued or fresh matter added to the lesson a new phenomenon supervenes which is the reverse of that initially experienced. The passage which a little while before was repeated faultlessly cannot now be repeated without mistakes. The attention wanders readily. Recollection becomes increasingly difficult, the consciousness has to be "flogged" into activity and finally excessive fatigue compels desistance from the task. The effects of the initial facilitation have not been undone, however, for a return to the task after an adequate interval for recuperation reveals the fact that the previous study has implanted memories which disappear from the field of consciousness in many instances only after a lapse of time comparable with the duration of life itself.

We meet, therefore, in the exercise of any given intellectual function, with two apparently contradictory facts. Performance *facilitates* the exercise of the function, and it likewise *depresses* the exercise of the function. We note, furthermore, that the facilitation and depression become evident at different periods of time, the former in the earlier stages of performance and the latter in its later stages.

Many hypotheses have been advanced by philosophers, psychologists and physiologists in the endeavor to imagine a mechanism which could account for the phenomenon of memory. The vast majority of the mechanistic hypotheses, which are the only ones of which we need

attempt the consideration, partake of the same general character; they assume that the previous repetition or performance has left some species of more or less permanent modification in the nervous system, and they vary only in the nature of this hypothetical modification.

Broadly speaking, the nature of this modification may be conceived in either of two ways which, for convenience sake, we may designate, respectively, the "static modification" and the "dynamic modification." The static conception, as developed especially by Munk and Ziehen, regards the "trace" or "image," which has been formed in the nervous system in consequence of some act or repetition, as consisting of some structural modification, some physical alteration, an alteration, in other words, in the distribution of cell-matter in space. The objections which may be and have been urged against this view are manifold. A purely physical alteration, namely the redistribution of preformed cell-material in space, would be something of the nature of a strain produced in response to some stress (= stimulus) which might be conceived of as mechanical, electrical, thermal or yet some other type of energy-change capable of inducing modifications of the physical state of matter. Now the remarkable **Persistence of Memories** proves that the "trace," whatever it may be, is rather permanent and only very slowly fades away. Indeed such investigations as those of Prince or Sidis would appear to indicate that a large proportion of memory-traces may persist in some measure throughout a lifetime. Of course, reinforcement of the trace by occasional "recollection," either conscious or "subconscious" may have occurred from time to time in the interval between the receipt of an impression and its emergence from consciousness under abnormal psychological conditions, such as those imposed by **Hypnosis**, at a much later period of life. Reinforcement of the trace by recollection cannot, however, be the general rule, for otherwise, as Sidis has pointed out, our entire mental life would be occupied in recollecting.

The memory trace, or at least some residual fragment of it, is therefore an extraordinarily persistent modification. The material of which the central nervous system is composed, however, is largely fluid or semifluid, and all our experience teaches us that a fluid cannot retain physical strains for any prolonged period; indeed it is this quality which enables us to recognize a fluid or a jelly and distinguish it from a solid.

A modification of the theory of Munk is that which was proposed by Lepine and Duval and has been very widely adopted by a certain school of neurologists and psychologists. This theory is based upon the demonstration by Cajal that the nervous system is divided, like other tissues, into distinct cell-units, or **Neurons**, which he regarded as being in contact with one another through the medium of their cell-processes or **Dendrites**, but not physically continuous with one another. It was assumed by Lepine that the formation of a new memory-trace in the nervous system was attributable to the formation of a new den-

drite-contact, while **Amnesia** or the phenomenon of forgetting represents the breaking of a contact previously established. To this view there attach most of the difficulties attendant upon Munk's hypothesis and, furthermore, as Meyer has very justly pointed out[1] the invocation of such hypothetical structural changes to explain the physical correlates of psychic phenomena must necessarily lead, sooner or later, to the invention of a metaphysical entity to keep the apparatus in order. Meyer expresses this difficulty as follows: "Why does the protoplasm stretch toward one neighboring neurone when the organism happens to be in one situation, toward another neurone when the organism is in another situation? General silence with the neurologists. But some psychologists had an answer ready. They brought in their *deus ex machina*. The Ghost does it. Consciousness, feeling, will, or whatever you call it, turns the bridge in the proper direction as the switchman turns the switch in a railway-yard." The cytological basis of this hypothesis has also been called severely in question since the investigations of Epathy, Bethe and others have demonstrated the existence of fine intercommunicating fibrils which, in many instances at least, establish anatomical continuity between adjacent dendrites.

The dynamic conception of the memory-trace, on the other hand, regards it as being formed by a chemical alteration of cell-material along the nervous path which was followed by the stimulus which is subsequently recalled. The superior generality and simplicity of this hypothesis is evident at once. It does not exclude the possible formation of a definite structure as the result of chemical change, on the other hand the persistence of memory traces is at once accounted for since, as we have abundant reason to know, chemical changes within living organisms may be as enduring as life itself.

We have seen (Chapter XVIII) that the rate of conduction of impulses in **Nerve-fibers** is conditioned partly if not wholly by physical changes which underlie the passage of the impulse. We infer this from the low **Temperature-coefficient** of conduction in peripheral nerve-fibers. In **Nerve-cells**, on the contrary, the passage of impulses is demonstrably accompanied by chemical changes. The temperature-coefficient for the conduction of impulses in the nerve-cells of the respiratory center and the cardiac ganglion, for example, is of the chemical order of magnitude. Furthermore, as Mosso has demonstrated, excitation of the cerebral cortex results in a pronounced disengagement of heat. Repeated attempts to demonstrate a similar evolution of heat in nerve-fibers in consequence of stimulation have failed. The processes which attend the conduction of impulses through nerve-cells, therefore, appear to be of a fundamentally different character from those which accompany the passage of impulses in nerve-fibers.

The effect of the chemical change which accompanies the passage of

[1] Meyer: Journal of Philos. Psychol. and Scientific Methods, 1912, **9**, p. 365.

an impulse through the central nervous system is to initially facilitate and ultimately retard the passage of subsequent impulses along the same path. The nature of the initial facilitation has been variously characterized. Thus Maudsley described it as the formation of a trace or thread of a deposit which is followed by the succeeding impulse, while Exner likened it to the "excavation of a channel," a hypothesis which is generally referred to as the **Canalization Hypothesis.**

In preceding chapters we have had frequent occasion to dwell upon a variety of chemical processes and not a few life-phenomena which display initial facilitation followed by retardation. These are the various processes or phenomena which are governed as to their speed by underlying **Autocatalyzed Reactions.** It is evident that if the passage of an impulse through the central nervous system were attributable to the occurrence of an autocatalyzed chemical reaction, the deposition of the products of this reaction along the path of the impulse would facilitate the passage of a subsequent impulse, while their accumulation in undue amount would constitute an impediment to the further occurrence of the reaction and therefore to the passage of subsequent impulses. The same mechanism thus accounts for both the facilitation and the fatigue which accompany the performance of functions involving the central nervous system.

Regarding the nature of the autocatalyst in this reaction we are of course completely in the dark in so far as any direct results of chemical analysis are concerned. We may, however, draw certain more or less probable inferences from our knowledge of the behavior of a particular part of the central nervous system, namely, the **Respiratory Center.** In this region we have a rhythmic passage of impulses of which the frequency is determined by the alternate facilitation and retardation of conduction which is brought about, as we have seen in a preceding chapter, by the presence of greater or lesser amounts of **Lactic Acid, Carbon Dioxide,** or other fatty or hydroxy fatty acids in the circulating fluids. Evidently, therefore, acids, or at least this particular class of acids, facilitate the passage of impulses through this if not through other regions of the nervous system. Now hyperactivity of the central nervous system results in the accumulation of acid substances in the brain, and we may with some probability infer that the normal activities of the central nervous system are accompanied to a lesser degree by the production of similar substances.

THE FATIGUE-PRODUCTS OF NERVE-CENTERS.

It has been pointed out by Mosso that the fatigue-products of **Nerve-centers** and those of **Muscle** are probably very similar in nature since mental fatigue is accompanied by signs of muscular fatigue and *vice versa.* Among the products of muscular activity two acids figure very largely, namely **Lactic Acid** and **Carbonic Acid,** and, if the products of muscular and of nerve-cell activity are similar, we should expect to

find that acids are set free in the central nervous system as a result of its activity or fatigue. The actual demonstration of an increase in acidity of the brain-substance as a result of prolonged excitation has proved difficult on account of the slightness of the change of hydrogen ion concentration which is involved, owing to the buffer-action of the tissues and tissue-fluids, and the technical difficulties, almost insuperable it would appear, which attend the utilization of adequate electrochemical methods of estimating the hydrogen ion changes in nervous tissues. We can, however, perceive the changed reaction of the brain after excessive stimulation by the employment of a simple indicator, provided, however, that instead of employing the change of color of the indicator as a sign or measure of acidity, we employ the change in its *solubility* in a solvent which is immiscible in water.

If to ten cubic centimeters of a concentrated (two per cent.) and very faintly acid solution of **Neutral Red** in water we add a single drop of tenth-normal potassium hydroxide the color of the solution does not perceptibly change, but nevertheless a great change is seen in respect to the lipoid-solubility of the neutral red if we shake up the original and the faintly alkaline solutions with **Ethyl Acetate**, from which any admixture of acetic acid has been previously carefully removed. On shaking up with the faintly acid solution of neutral red the ethyl acetate remains absolutely colorless, while on shaking it up with the faintly alkaline solution the ethyl acetate layer is stained deep yellow. In two ways the indicator is rendered more sensitive by this method; in the first place a trace of the yellow modification of neutral red, which would be invisible in watery solution owing to the great excess of the red modification, is removed by the ethyl acetate and thereby rendered visible. In the second place, let us suppose that the **Coefficient of Distribution**:

$$\frac{\text{concentration in lipoid layer}}{\text{concentration in aqueous layer}}$$

is 100 : 1 for the yellow modification of neutral red, and zero for the red modification. Then at any given concentration "b" of hydroxyl ions, if "y" be the concentration of the red modification and "x" that of the yellow modification:

$$x = kf(b)y$$

where "k" is a constant and f (b) is some function of the alkalinity not necessarily known or defined. Now let this solution be shaken up with ethyl acetate, and let the concentration of the yellow modification in the watery layer now be "x," while that of the red modification is "y," and that of the yellow modification in the ethyl acetate layer is "x_2," then we have:

$$\begin{aligned} x_1 &= kf(b)y_1 \\ x_2 &= 100x_1 \\ x_2 &= 100kf(b)y_1 \end{aligned}$$

that is, the concentration of the yellow modification in the lipoidal layer (ethyl acetate) is 100 times its concentration in the watery layer and, provided f (b) were a linear function, it would be the same concentration as that which would be produced in the watery layer by 100 times the concentration of hydroxyl ions. In other words the sensitiveness of the indicator is multiplied by the distribution-coefficient of the lipoid-soluble modification between the two immiscible solvents. In addition to this there is, as has been stated, an apparent or "physiological" increase in the sensitiveness of the indicator due to the physical separation of the two colors.

Two frogs may be taken and a powerful stimulus applied to the skin of one of them by means of an induced current for a prolonged period (half an hour) while the other is left undisturbed. The brains of both animals are then rapidly removed, divided longitudinally and the two parts of each placed in a two per cent. neutral aqueous solution of neutral red for from four to five minutes. The two brains are then removed from the neutral red solution at exactly the same moment and dropped into neutral ethyl acetate.

Within five or ten minutes there is seen to be a distinct difference between the colors of the cut surfaces of the two brains. The cut surface of the brain which has been stimulated remains deep red, but the indicator diffuses out of the unstimulated brain, and the depth of color diminishes until it is only pink. The differences in color increase for some time, and in some instances after the lapse of an hour the unstimulated brain may be almost colorless, owing to extraction of the dye by the ethyl acetate, while the stimulated brain retains a reddish pink hue. Evidently the stimulated brain behaves like a faintly acid aqueous layer, the unstimulated brain like a faintly alkaline aqueous layer. The development of acid as a fatigue-product of nerve-centers may thus be clearly inferred.

It might be imagined that in this experiment the increased acidity of the brain may be apparent and not real, being due to acids carried to the brain by the blood from the tetanically contracting muscles of the stimulated frog. It has been shown by Gobau, however, that precisely the same result is obtained if the frog employed for stimulation is previously curarized, in which case the muscles are immobile.

Acids are therefore produced in the brain in consequence of its activity and in the respiratory center, if we may take this area as representative of the whole, certain specific acids accelerate the passage of impulses through it. We have thus experimental verification of the view that central nervous phenomena are self-catalyzed. The catalyzer which is responsible for the formation of **Memory-traces**, however, is not probably any substance so simple as lactic or carbonic acids, which as we have seen, are stimulants of the respiratory center. These substances are so soluble in water that they would very rapidly be washed out of the nervous tissues and the persistence of memory-traces would be inexplicable. It is more likely that we have here to

deal with a colloidal fatty acid which is deposited along the path of an impulse and remains to accelerate or, if it is in excess, to retard a subsequent impulse.

THE APPLICATION OF THE FORMULA OF AUTOCATALYSIS TO CENTRAL NERVOUS PHENOMENA.

The time-relations of any **Voluntary Movement** are primarily governed by events which occur in the central nervous system. This may readily be inferred from the fact that it requires, not a single impulse or stimulus to produce any coördinated movement, but a stream of impulses

FIG. 46.—Photograph of a drawing-board specially constructed to record the time-relations displayed in the execution of a simple volition (the drawing of a straight line).

which must be maintained throughout the duration of the act which is performed. A single stimulus, when applied to voluntary or striated muscle, only produces a single rapid twitch; a prolonged tetanic or semitetanic movement such as that involved in the performance of any muscular exertion is only possible to evoke by a rapid succession of stimuli. Moreover the performance of a coördinated muscular act such as that of bending the arm, involves a simultaneous discharge of stimulatory impulses to the flexor, and inhibitory impulses to the opposing extensor muscles of the limb.

The time-relations of a simple voluntary movement, such as that implied in drawing a straight line with a pencil upon a board, may be accurately investigated by a method which was originally proposed by

Loeb and Korányi. A drawing-board is made up of alternate strips of metal and wood and is carefully polished so that the junction of the strips is as nearly as possible indistinguishable to the touch. The metallic strips are connected together in a circuit which includes a signal-magnet and a metallic pencil. When a line is drawn upon the board with the metallic pencil the moment at which the pencil touches or leaves a metallic strip is indicated upon a cylinder of smoked paper by the signal-magnet (Fig. 46). In this way the length of line traversed at any instant in the entire process can very readily be determined. It is convenient to provide a ruler, firmly affixed to the board, to guide the movements of the pencil and to start the line from a check or crotch in the ruler, the position of which on the board with reference to the nearest succeeding metallic strip has been accurately determined.

When the relationship between the time and the extent of movement in drawing a straight line is investigated in this way it is found that the **Autocatalytic Formula:**

$$\log \frac{x}{a - x} = ka(t - t_1)$$

applies with remarkable accuracy, "a" being the total length of the line, "x" the length of line drawn at time "t" after the motion of the pencil first began, and "t," the time taken to reach the middle of the line. The following is an illustrative result:

Subject R.M.M.♂ Formula: $\log \frac{x}{31.0 - x} = k\,(t - 38.75)$

x inches.	t 1/100 secs.	k
1	10.00	0.051
2	15.50	0.050
3	17.50	0.046
4	21.00	0.047
5	22.50	0.044
6	25.00	0.045
7	26.00	0.042
8	27.50	0.041
9	29.00	0.040
10	30.75	0.040
11	32.00	0.038
12	33.50	0.038
13	35.00	0.038
14	36.50	0.037
15	38.00	0.037
16	39.50	0.037
17	40.75	0.042
18	42.00	0.043
19	43.50	0.042
20	45.00	0.041
21	47.00	0.039
22	48.25	0.041
23	50.00	0.041
24	52.00	0.044
25	53.75	0.041
26	55.50	0.043
27	57.50	0.044
28	59.50	0.049
29	61.75	0.050
30	66.00	0.054

The values in the third column are, as would be required by the formula of autocatalysis, almost constant. The performance of this particular type of central nervous activity is therefore autocatalyzed.

Turning now to the much more complex phenomenon of **Memory** we are in possession of quantitative data which have been most elaborately compiled by the psychologist Ebbinghaus. The method which he employed was to read and reread a series of meaningless syllables at a definite rate, 0.40 seconds being expended in the perusal of each syllable. The data recorded are the numbers of repetitions which were found to be necessary to attain the perfect memorization of the given number of syllables in the series. Hence the time in seconds which was employed in learning each series was $0.4 \times n \times r$ where "n" and "r" were the number of syllables in the series and the number of repetitions respectively. Excepting in the case of the first observation (that is, the number of syllables learnt in a single repetition) the syllables were read in conjunction with a sufficient quantity of other material to make the total length of each period of reading approximately the same. The following were the results obtained:

Number of syllables.	Number of repetitions.	Time in seconds.
7	1.0	2.8
12	16.6	79.7
16	30.0	192.0
24	44.0	422.4
36	55.0	792.0

If we apply to these results the formula of autocatalysis, calling "a" the maximal number of syllables which Ebbinghaus could have memorized by any number of repetitions, "x" the number actually learnt or the extent of the trace or deposit formed in time "t," and "t," the time consumed in learning half the maximal number, we find that the following equation most nearly expresses the results:

$$\log_{10} \frac{x}{43.6 - x} = 0.001468 \quad t - 0.526$$

In the following table the experimental values of "x" and those calculated from the formula are compared:

Time in seconds	x (observed)	x (calculated).
2.8	7	10.1
79.7	12	12.2
192.0	16	15.8
422.4	24	24.2
792.0	36	35.4

The only deviation of significant magnitude is that between the observed and calculated numbers of syllables which may be learnt in a single repetition. This, however, may most probably be attributed to the conditions under which this number was determined, differing as they did, by the non-inclusion of other reading matter, from the conditions which pertained in the remaining observations.

The view that the formation of the **Memory-trace** is due to an autocatalyzed chemical reaction, therefore, not only enables us to interpret some of the most striking qualitative phenomena of intellectual processes, but also to predict their quantitative alteration with successive repetition. The quantitative data obtained by Ebbinghaus are among the most readily interpretable and at the same time accurate measurements of this kind which we possess, but a variety of measurements which have been made on the rate of learning by telegraph-operators, typists, and so forth, all yield "curves of learning" which very strikingly resemble the curve which represents the progress of an autocatalyzed chemical reaction, and in some cases, it appears, two or more of such curves may be superimposed to yield "cycles of learning" just as we have cycles of growth in a growing organism.

SLEEP.

The various theories of sleep which have been proposed are no less numerous than those which have been propounded to account for the phenomenon of memory. A **Vasomotor Theory of Sleep** has been advanced by Howell, who considers that it is attributable to cerebral anemia, due to a diminished blood-supply to the brain, following the general fall of arterial pressure which accompanies sleep. While this may very possibly be a contributing factor to the phenomenon of sleep, yet, on the other hand, it is at least equally conceivable that the vasomotor-phenomena which accompany sleep are merely secondary manifestations of the processes which induce sleep, and that the actual onset of sleep is due primarily to other factors. The close connection of sleep with **Fatigue** on the one hand, and with the absence or monotony of **Sensory Stimulation** on the other, indicates very clearly that a condition of the nervous tissues consequent upon prolonged activity is a potent factor predisposing the central nervous system to the relatively suspended activity of sleep.

The accumulation of **Fatigue Products** in the brain, when it has exceeded the amount which causes maximal facilitation of the passage of nervous impulses, begins to retard the passage of impulses, and this retardation increases with the degree of accumulation. With continued wakefulness, as many observers have pointed out, the **Threshold of Sensory Stimulation** rises. A stronger stimulus than usual is required to traverse the clogged and overloaded channels, and consequently the environment, by exclusion of the countless slight fluctuating impressions which lend variety to our surroundings, becomes more and more monotonous, fewer and fewer "channels" of the brain are traversed by impulses, larger and larger areas become quiescent through lack of traversing stimuli, until finally sleep supervenes, and the whole of the brain except those portions, chiefly in the medulla, which are vital to the maintenance of the circulation and respiration, and some

detached fragment which may be occupied in weaving dreams, has subsided into quiescence.

It is the variety of our environment and the intensity of rapidly succeeding sensory impulses which keep us awake, by forming new "channels" which intersect with other channel-systems, *i. e.*, arouse "associations" and keep up a continuous activity over the whole area of consciousness. If the **Field of Consciousness** is limited, either by fatigue or by the limitation of incoming sensory impressions, one group after another of channel-systems or interconnected memory-traces sink into quiescence until only the least fatigued or the most intensely stimulated channels are awake. When the stimulation is nowhere sufficient to rise above the threshold of consciousness, we have sleep, but where the stimulation is intense, and yet excessively circumscribed, we have the condition of **Hypnosis**. The extraordinary vividness of the impressions which are formed under hypnosis is due to the isolation of these impressions and to the fact that for the moment the brain is, for all effective purposes, limited to and circumscribed by the areas which are directly stimulated.[1] Inhibitive and conflicting impressions are temporarily in abeyance.

The customary method by which we recollect past events is the **Association** of a present event with an incident which recalls the past. In other words a stimulus of the present moment happens to traverse a previously formed system of trace-deposits. If, however, only a small portion of the brain be active the chance of a subsequent impulse traversing it must obviously be less than when the area of stimulation is larger. The cutting off of sensory impressions in sleep and the diminution of the extent and variety of "canalization" or trace-formation throughout the upper portion of the central nervous system which accompanies sleep is therefore conducive to **Amnesia** or lack of ability to recollect the intellectual events which occur under these circumstances. This fact is well illustrated by phenomena which frequently attend the onset of sleep. A certain sequence of ideas arises in the consciousness—we think of it, as we say, dreamily—then suddenly this train of ideas vanishes and another takes its place, and we find that we cannot recollect the first. This amnesia is occasionally so surprising in itself that the wonder of it excites us to the extent of awakening. So the cessation of canalization in one trace-system leads, by the blocking off of impulses, to its cessation in an adjacent system, and amnesia spreads over a wider and wider area, until finally sleep supervenes. The fabric of intercommunicating trace-systems which constitutes the waking consciousness shows larger and larger rents of amnesia, the fragments of the fabric are less and less bound together, until at last the entire fabric seems to be blotted out, or one

[1] The impressions received during hypnosis are usually separated from the waking impressions by a gap of amnesia, but during the actual period of hypnosis the extraordinary vividness of the impressions received is testified by the almost automatic response of the body to commands or suggestions which are received in this condition.

shred may remain, as in a dream, to be faintly recalled or completely forgotten in awakening, according to whether or not our customary waking perceptions (traces) traverse the point of union of the dream-shred with the whole fabric of the reawakened consciousness.

That the onset of sleep is in reality due to the accumulation of **Fatigue-products** which are washed out during the period of quiescence by the circulatory fluids, has been very strikingly demonstrated by Piéron. This observer has shown that if the blood-serum or **Cerebro-spinal Fluid** of a dog which has been kept awake for an abnormal period be injected directly into the fourth ventricle of the brain of a normal dog, even if this latter animal has recently slept, it falls at once into a profound slumber. The effect is much greater if cerebro-spinal fluid or the fluid from the ventricles of the brain is employed than if blood-serum be used. Frequently with blood-serum nothing more than a moderate somnolence is elicited, whereas when cerebrospinal fluid is employed the slumber which is induced may be so profound that the animal will remain asleep in any attitude in which he may be placed. Piéron has made many interesting observations upon the chemical nature of this sleep-inducing substance, or **Hypnotoxin** as he designates it. He finds that it is destroyed by heating to 65° C. and by oxidation, is precipitable or coagulable by alcohol and is non-diffusible. It is evidently, therefore, a colloidal substance of some complexity, and chemically unstable.

THE FADING OF MEMORY-TRACES.

It is a matter of common experience, and a fact which has been experimentally verified, that a person who has been deprived of sleep beyond the normal period of wakefulness does not require the full sum of the periods of sleep which he has lost in order completely to recover from his desire to sleep. We must therefore conclude that not only do **Fatigue-products** disappear from the brain during sleep but, furthermore, that they disappear the more rapidly the greater their concentration.

We have seen that the initial effect of the fatigue-products of the central nervous tissues is to cause facilitation of the passage of nervous impulses and the formation of **Memory-traces.** The phenomenon of forgetting must therefore be essentially of the same nature as the phenomenon of refreshment by sleep, *i. e.*, it must consist in (or depend upon) the disappearance of the products of their functional activity from certain nerve-tracts.

Ebbinghaus has carried out a number of excessively painstaking investigations upon the rate at which meaningless syllables which have once been learned by heart are forgotten. Ebbinghaus was his own subject. Series, each consisting of thirteen meaningless syllables, were read and reread in such a manner that each syllable was presented to the senses for a period of 0.41 seconds at each repetition. When it was found just possible to completely recall the series correctly, the total time ($= t_1$) consumed in memorizing the series was noted.

After the lapse of certain definite periods of time the series were relearned, and the time ($= t_1 - t$) necessary to relearn them was also noted. Then the difference ($= t$) represented the time saved by the previous repetitions, or in other words the time which would be consumed in learning that proportion of syllables which was remembered. The percentage $\frac{t_1-t}{t_1} \times 100$ was employed by Ebbinghaus (and has been employed by his successors in this field of investigation) as the most convenient measure of the extent of forgetting. It is, of course, not actually equivalent to the amount of memorized material which has been forgotten, for the time required to memorize syllables is, as we have seen, not proportional to their number. Nevertheless the outline of the relationship between the time which has elapsed since the material was learned and the amount of material forgotten is sufficiently clearly revealed by the successive values of $\frac{t_1-t}{t_1} \times 100$ noted by Ebbinghaus to show that this phenomenon, like that of refreshment by sleep, occurs most rapidly in the beginning, when the mass of deposit undergoing destruction or dispersal is greatest. The following were the results obtained by Ebbinghaus—the time being reckoned from the end of the first period of learning to the end of the second.

Time in hours.	$\frac{t_1-t}{t_1} \times 100$
0.33	41.8
1.00	55.8
8.80	64.2
24.00	66.3
48.00	72.2
144.00	74.6
744.00	78.9

The negative acceleration of this process is extraordinarily high, for although over 55 per cent. of the time saved by the first period of learning is lost in one hour, yet during the succeeding twenty-three hours only 9 per cent. more is lost. In other words the **Velocity of Forgetting** decreases very rapidly with the passage of time; it never, under normal conditions, undergoes any increase in rapidity with time. The process of forgetting is therefore essentially different in mechanism from the process of memory-formation.

It is very improbable that the fading of a memory-trace can be due to chemical changes in the substance forming the trace, for no chemical reactions are known which diminish so greatly in rapidity with time, continue to proceed, and yet do not attain completion for such prolonged periods as the memory-traces persist. A reaction which was 55 per cent. completed in one hour would either have ceased before twenty-four hours, or else would be much more than 66 per cent. completed. A chemical reaction, to display such extraordinary falling-off

in velocity with time, would have to be polymolecular, *i. e.*, involve a large number of *simultaneously* reacting molecules, and polymolecular reactions do not actually occur, or rather they take place in successive monomolecular, bimolecular or trimolecular stages.

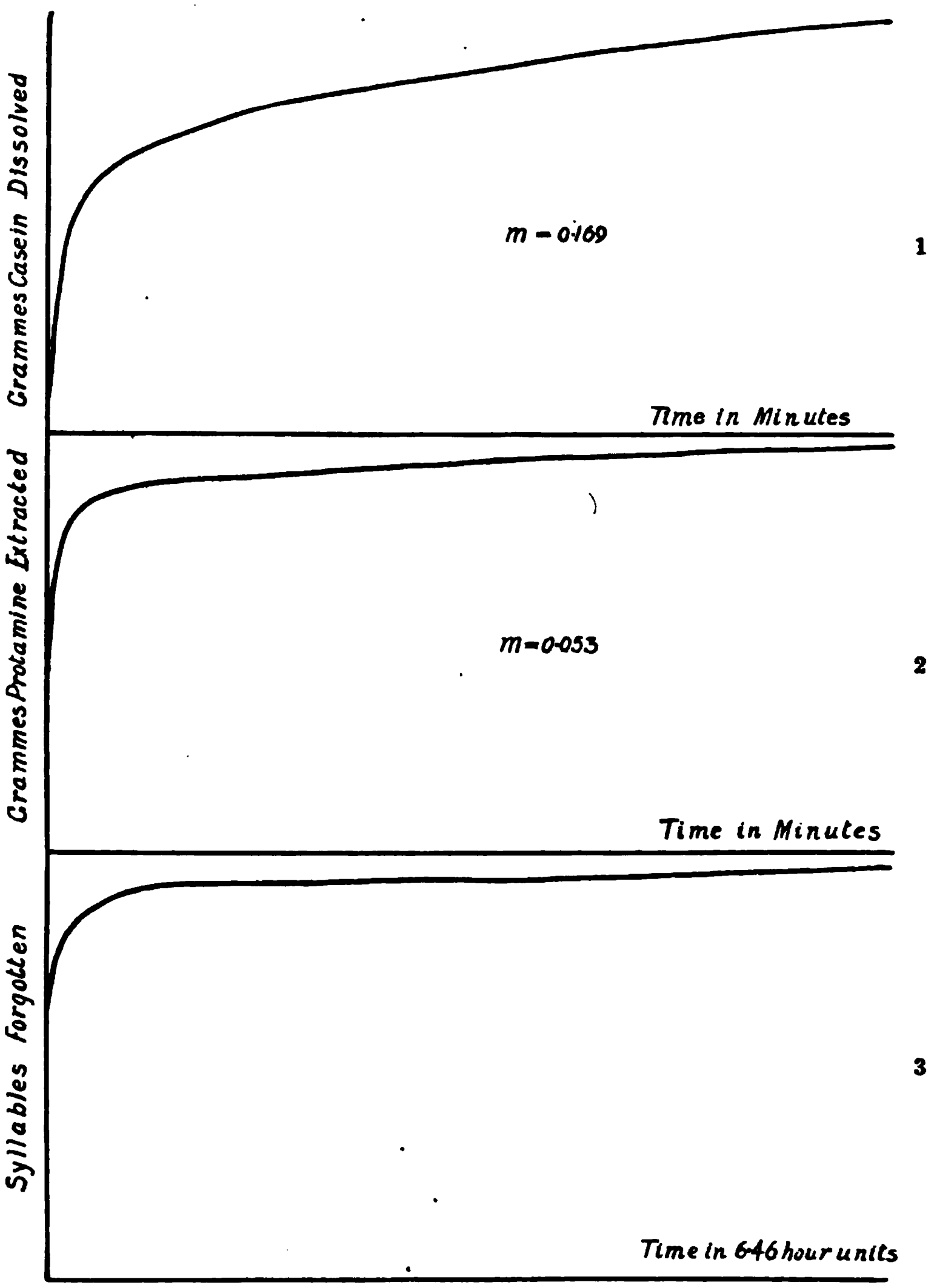

FIG. 47.—Curves illustrating the analogies between the fading of a memory trace, the extraction of protamine from spermatozoa by acid and the dissolution of dried casein by dilute alkali.

If, however, we compare the curve of forgetting with the curve which expresses the rate of issuance of a colloid (or possibly of a crystalloid) from a colloidal into a fluid menstruum, we cannot fail to recognize

at once their essential similarity. In the accompanying figure (Fig. 47) curve 1 represents the rate of issuance of potassium caseinate from suspended **Casein** particles into dilute potassium hydroxide solution; 2 represents the rate of extraction of **Protamine** (salmin) from dried salmon-spermatozoa by dilute hydrochloric acid; and 3 represents the **Curve of Forgetting**, as illustrated by the results of Ebbinghaus cited above. Comparing these curves it is evident that by a suitable modification of parameters any one of them might be employed in place of the others to illustrate the processes which they severally depict, and that each of them represents the time-relations of a process in which the negative acceleration is so marked as to forbid its representation by any known chemical reaction-formula, or by the similar formulæ which represent the diffusion of crystalloids in fluid media. It has been found that the issuance of a protein (and therefore, probably of other colloids) from a colloidal menstruum is governed primarily by **Capillary Forces** so that the time-relations of the washing-out process are similar to those exhibited in the rise of a fluid in a capillary tube or of a liquid in a column of sand or a strip of filter-paper. We may infer that the fading of a memory-trace is attributable to some similar phenomenon and may not improbably be due to the washing out of a colloidal substance, which forms the memory-trace, by the circulating fluids. This would explain at once the rapidity of the initial stages of forgetting and the extraordinary persistence of the last traces of the memory-deposit, for complete extraction of a colloid from a colloidal menstruum by an external liquid is a matter, not of hours, but, as may be computed by exterpolation from actual measurements, may actually require the lapse of periods of time which are vastly in excess of the total duration of the life of man.

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PART V.

THE PRODUCTS OF TISSUE-ACTIVITY.

CHAPTER XXII.

THE WASTE-PRODUCTS.

THE CARBONACEOUS WASTE-PRODUCTS.

The chief carbonaceous waste-product is, of course, **Carbon Dioxide.** Only a trifling proportion of the excretion of carbon dioxide takes place through the urine, feces and sweat, the lungs playing the preponderating part in accomplishing the elimination of this product. The total production of carbon dioxide in twenty-four hours varies with the quality and quantity of food ingested, with the quantity of muscular work performed, and with the rate or loss of heat from the body, but in an adult male doing moderate work it may be estimated in round numbers at four hundred liters at ordinary temperatures and atmospheric pressure.

The carbon-dioxide output is derived from the oxidation of the carbon in the metabolized foodstuffs. It arises, therefore, in consequence of the absorption of oxygen by the tissues. Carbon dioxide is, however, not the only oxidation-product of cellular activities, and hence the carbon dioxide which is given off by an animal is rarely the molecular equivalent of the oxygen which is absorbed in the same period. The ratio: $\frac{CO_2 \text{ discharged}}{O_2 \text{ absorbed}}$ is termed the **Respiratory Quotient** and it varies in a very characteristic manner with the nature of the ingested foodstuffs. Thus the carbohydrates contain a greater proportion of oxygen than any of the other foodstuffs, the oxygen being, in fact, molecularly equivalent to the hydrogen which they contain. The hydrogen in a carbohydrate may, therefore, be regarded as having been completely oxidized beforehand, and the carbohydrates behave, so far as the absorption of oxygen and evolution of carbon dioxide are concerned, as if they consisted of pure carbon and underwent the reaction:

$$C + O_2 = CO_2$$

hence the respiratory quotient for the oxidation of pure carbohydrates is equal to unity. This probably represents the maximal value of the respiratory coefficient which may be obtained with normal animal tissues. Figures in excess of this which have occasionally been observed have been attributed by some observers to the formation in the tissues of fat from carbohydrates with the liberation of carbon dioxide:

$$\underset{\text{Glucose.}}{4C_6H_{12}O_6} + O_2 = \underset{\text{Palmitic acid.}}{C_{16}H_{32}O_2} + 8CO_2 + 8H_2O$$

Respiratory quotients in excess of unity have been observed in hibernating animals immediately prior to their winter-sleep, and in animals and birds fed with an enormous excess of carbohydrates.

The respiratory quotient for the oxidation of **Fats** is necessarily much lower than it is for carbohydrates, since the fats do not contain more than about one-sixth of the oxygen which is required to convert the hydrogen which they contain into water. An important proportion of the absorbed oxygen is therefore excreted in the form of water, and the carbon dioxide which is discharged from the body falls very much short of the molecular equivalent of the oxygen absorbed, the respiratory quotient for the ordinary dietary fats being 0.71. The **Proteins** contain about half the oxygen needed to oxidize their hydrogen and the respiratory quotient is intermediate between the value for carbohydrate and fats, namely 0.81. The respiratory quotient for **Alcohol** is lower even than for fats, namely 0.67.

From these considerations it is evident that the value of the respiratory coefficient must be capable of yielding important information as to the particular class of foodstuffs which is being utilized for the performance of a given function. Thus for man, under ordinary conditions of work and nourishment, the respiratory quotient lies between 0.8 and 0.9, but when hard **Muscular Work** is being performed it rises and may even approach the ideal value of unity for the oxidation of carbohydrates. Part of this rise, especially during the initial stages of a work-experiment, or in experiments occupying only a short period, may possibly be ascribed to the "washing out" of carbon dioxide accumulations from the tissues by the more rapid respiratory and cardiac movements. It must be recollected, however, that the rapidity of the respiratory movements in exercise is conditioned by the *enhancement* of the carbon-dioxide content of the blood, so that but a slight proportion of the increased carbon-dioxide output during exercise can justifiably be attributed to the increased ventilation of the body, and, furthermore, the effect of muscular work upon the respiratory quotient endures for a long period, until in fact, the carbohydrate-reserves have been so far depleted that we may surmise that other foodstuffs are now being utilized for the production of muscular energy. The rise of the respiratory quotient during the performance of muscular work therefore affords us confirmatory evidence of the view that muscular

energy is derived, in the first place, from the oxidation of carbohydrates.

On the other hand, in **Starvation**, the respiratory quotient falls to a value intermediate between that characteristic for the oxidation of fats and the value for proteins, for in starvation the carbohydrate reserves are quickly depleted, and thereafter the energy which is dissipated by the body is derived from the oxidation of the fat reserves and the tissue proteins.

Extraordinarily low values of the respiratory quotient have occasionally been obtained with **Hibernating Animals** during their winter-sleep. Thus Pembrey obtained figures as low as 0.25 with hibernating dormice. · For hibernating bats Hári obtained higher figures, but even these values were generally less than the normal value for the oxidation of pure fats. The origin of these low values has been the subject of numerous surmises. It appears to be incontestable that they represent incomplete oxidations, which do not proceed so far as to result in the formation of carbon dioxide. A question much more difficult to decide, however, is whether the excess of oxygen intake over carbon-dioxide output in the winter-sleep is stored in the animal's tissues, or excreted in the form of compounds other than carbon dioxide. It was at first supposed that the oxygen excess was stored in the tissues in the form of partially oxidized foodstuffs, as, for example, carbohydrates derived from fats. It has been pointed out, however, that the total accumulation of oxygen throughout the duration of the winter-sleep would necessitate the production of a quantity of carbohydrate far in excess of the total carbohydrate-content of the animals under any conditions. It has been ascertained that the urine of hibernating animals contains notable quantities of products of incomplete oxidation such as **Lactic Acid**, and it is probable that a considerable proportion of the excess of absorbed oxygen is excreted in the urine in these forms.

Not only does the ratio of carbon-dioxide evolved, to oxygen absorbed, rise during the performance of muscular exercise, but the total carbon-dioxide output increases in direct proportion to the work performed. This has been shown in a very striking manner by the experiments of Johansson who first measured his carbon-dioxide output per hour at rest and then during the performance of the **Muscular Work** involved in repeatedly lifting a weight. He found that his carbon-dioxide output rose to the value

$$CO_2 = Np + q$$

where "q" was the output at rest, "N" the number of times the weight was lifted and "p" the increase in output induced by lifting the weight once.

The effect of the **Temperature** of the environment upon the carbon dioxide output is opposite in cold-blooded and warm-blooded animals.

In cold-blooded animals, in which the temperature of the tissues approximates to that of the environment, the rate of oxidations is increased, as might be expected, by a rise in external temperature, and the carbon-dioxide output is even more than proportionately increased, since the respiratory quotient generally undergoes a slight rise with temperature also. This is illustrated by the following experiments of C. J. Martin on the carbon dioxide output of the Australian Lizard *Cyclodus gigas*.

Temperature of the air.	Temperature of the animal.	CO_2 output per kilogram and hour, mg.
5.	5.5	13
9.	9.2	42
15.	15.2	53
20.5	20.4	55
25.	24.5	64
30.	29.3	78
35.	34.8	97
39.	38.5	292

The effect of rising temperature upon the carbon-dioxide output of warm-blooded animals is, within certain limits, the reverse of this. The **Body-temperature** of the warm-blooded animals varies but slightly with the temperature of the environment and this uniformity of temperature is secured by a number of coöperating factors, among which the radiation of heat from the surface of the body, the loss of heat by the latent heat of evaporation of perspiration, and the adjustment of the production of heat by the oxidations of the body to the need for heat to maintain the normal temperature of the tissues. The increase of metabolism which low temperatures induce in the warm-blooded animals is probably brought about, in part at least, by the stimulation of the skin by cold air inducing reflex movements, such as shivering or reflex alterations of muscular tone which necessitate an enhanced combustion of carbohydrates with the performance of a minimum of external work.

The regulation of the temperature of the body between the normal "comfortable" temperature-limits of the environment is mainly brought about by the modification of the purely physical factors of radiation and evaporation which govern the rate of loss of heat from the body. Below the external temperature of 20° C. (68° F.), however, the "chemical regulation" of the bodily temperature becomes an exceedingly important factor, the rate of metabolism rising continuously, and considerably with falling temperature. Above 30° C.–35° C. (86° F.–95° F.) the effect of temperature upon the oxidations of the body varies with the humidity of the air. The greater part of the heat-loss at these high temperatures is accomplished through the evaporation of perspiration, and if the humidity of the atmosphere be so great as to interfere with this method of heat-dissipation the regulatory mechanisms of the body become inadequate, the bodily temperature rises and with it the rate of oxidation and the total output of heat, just as it

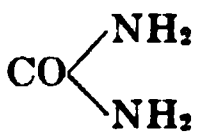
would in cold-blooded animals. It is to this that the exhausting effects of the **Tropical Climate** are to be referred. A temperature of 86° F. in an atmosphere saturated with moisture is almost unbearable, and physical work is, for Europeans at least, an impossibility, while a temperature of 110° F. in a perfectly dry atmosphere can be endured, and even a considerable amount of physical work performed, without any exceptional discomfort, by persons in normal health whose temperature-regulating mechanisms are in good order.

Among the remaining carbonaceous waste-products under normal physiological conditions may be enumerated **Methane** which is derived from bacterial fermentations in the intestine but is exhaled mainly through the lungs. The quantity of methane produced by carnivora and animals which subsist upon a mixed diet, such as ourselves, is normally a very small proportion of the total carbon output, but in herbivora it may become very appreciable. **Oxalic Acid** is regularly found in normal urine in very small amounts, the normal excretion being about 0.02 grams in twenty-four hours. Its origin is unknown. As it is a frequent product of bacterial fermentations it may have an alimentary origin, and, again, the administration of sodium oxalate leads to the appearance of the unchanged oxalic acid in the urine, and a number of foodstuffs, particularly fruits and vegetables, contain oxalates which would therefore appear in the urine. On the other hand, the output of oxalic acid continues on a pure protein diet and, on a normal diet, is stated to be enhanced by the administration of considerable quantities of gelatin, so that we may conjecture that the urinary oxalic acid is in part produced by the metabolism of the tissues. The output of oxalic acid is also stated to be increased in diabetes.

Lactic Acid is only found in the urine in partial asphyxia, or after the most extreme muscular exertion; its appearance in the urine indicates imperfect oxidation of carbohydrates or else extraordinarily excessive production by the muscular tissues.

THE NITROGENOUS WASTE-PRODUCTS.

Of the various nitrogenous waste-products, **Urea**:



is quantitatively the most important. The daily output of this substance varies with the quantity of protein which is ingested, but for the adult man subsisting upon a mixed diet the daily excretion is about thirty grams, for a woman somewhat less. This corresponds to from 84 to 90 per cent. of the total nitrogenous output.

The quantity of urea which is excreted varies directly with the quantity of protein ingested. We have seen in preceding chapters that animal tissues do not store up proteins and that their storage-capacity

for amino-acids is limited. The excess of amino-acids absorbed from the intestine is converted into urea by a series of steps which we are about to discuss, and this is excreted promptly in the urine. On the other hand the excretion of urea upon a diet low in proteins, but abundant in fats and carbohydrates, may actually be less than in starvation, because the fats and carbohydrates spare the tissue-protein from destruction for the production of the energy which is dissipated by the body.

The question of the region of the body in which urea originates has been the subject of a great many investigations. Since it is so prominent a constituent of urine, the kidneys naturally fall first under suspicion of being the organs in which the manufacture of this material takes place. This possibility has been the subject of experimental inquiry by a number of investigators. If the kidneys produced urea to the extent of an important proportion of the total output, then excision of the kidneys should lead to the disappearance of urea from the body, or at any rate should not lead to its accumulation. If, however, the kidneys simply eliminate urea which is produced primarily by other organs, then excision of the kidneys should lead to the accumulation of urea in the organs and tissue fluids. This is what actually occurs, and the accumulation of urea under these circumstances and in conditions involving inefficient excretion by the kidneys, as in **Nephritis**, has been repeatedly established.

We must therefore look elsewhere than to the kidneys for the main source of the urea which they excrete. From a variety of different experimental results we can definitely affirm that the **Liver** plays a very important role in the production of urea; whether it is the exclusive source of this substance or not cannot be regarded as definitely established, but a very large proportion of the total output originates in this organ. Thus if blood be perfused through the various organs in such a manner that the same blood passes without renewal through the tissues over and over again, no accumulation of urea in the blood is noted in the case of the kidneys or of muscular tissues, but a very pronounced accumulation occurs in the blood which is perfused through the liver.

The portal vein, which carries the blood containing absorbed foodstuffs from the alimentary wall to the liver, runs parallel with, and very close to, the inferior vena cava. By making an incision in the adjoining sides of these veins and sewing the edges together, an operation which is known as **Eck's Fistula**, the portal circulation is short-circuited and the blood from the intestine, with its load of food-products, no longer passes through the tissues of the liver. The liver is, however, still nourished by the circulation from the hepatic artery. Animals upon which this operation has been performed will survive for prolonged periods, and it was found by Pawlow and Nencki that in such animals the urea excretion is greatly diminished while the ammonia excretion is very considerably increased; in other words that ammonia

to a certain extent takes the place of urea in the urine of such animals. Confirmatory evidence is supplied by the effects of degenerative changes of the liver upon the urea output. In cirrhosis of the liver and in the liver-degeneration which is induced by **Phosphorus-poisoning** there is a decided diminution of the urea output, and a concurrent increase in the ammonia output.

It is impossible to settle this question by extirpation of the liver in mammals, since they do not survive the operation for a sufficient period to permit observation of the excretory products. In birds however, this severe operation may be performed without immediately fatal results. The birds do not, it is true, survive the operation for more than about twenty-four hours, but the time during which they live is sufficient to enable us to ascertain the effect of the removal of the liver upon the excretory products. Unfortunately urea is not the normal end-product of protein catabolism in birds; its place being taken by **Uric Acid**, which forms from one-half to three-fourths of the total nitrogenous output. However, the uric acid which is excreted by birds is undoubtedly the physiological equivalent of urea. In fact when urea is administered to birds it is excreted in the form of uric acid, so that were the tissues of birds to form urea it would nevertheless be excreted in this form. The effects of extirpation of the liver in geese were investigated by Minkowski, with the following results:

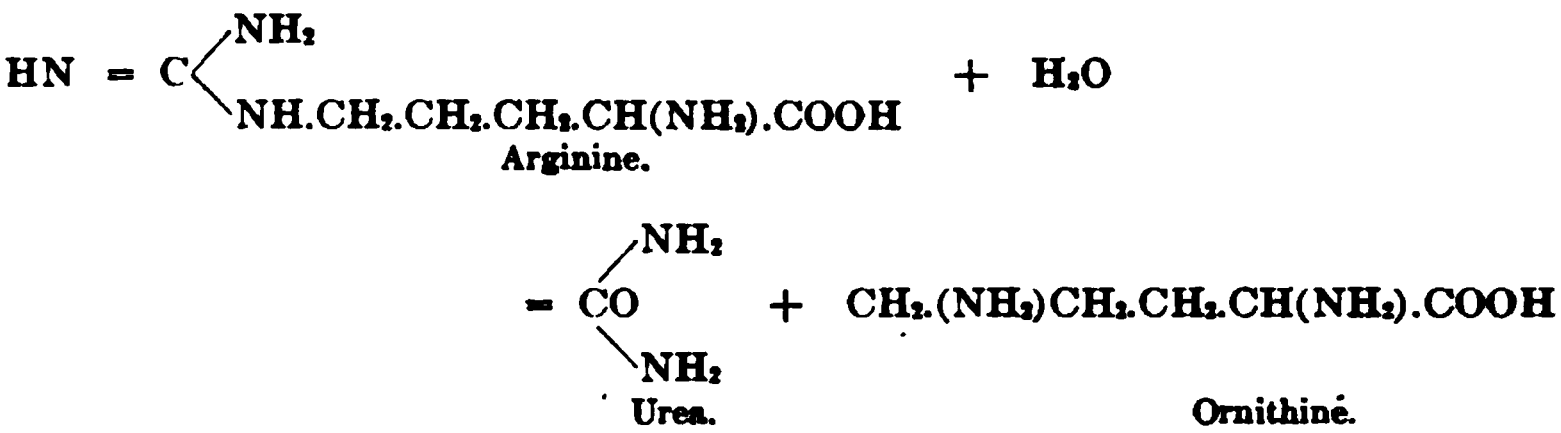
	Per cent. of total nitrogen in the form of: Uric acid.	Ammonia.
Before extirpation	60 to 70	10 to 18
After extirpation	3 to 6	45 to 60

These results are decisive, and the origin of at least ninety per cent. of the uric-acid output in birds must be in the tissues of the liver. Taking all of these different experiments together, therefore, and recollecting that the uric-acid excretion of birds is the physiological equivalent of the urea output of mammals, we are justified in inferring that the liver is a predominant, if not the sole source of the urea output of mammals. Nevertheless some urea output continues in animals which have an Eck fistula, even when the hepatic artery is also ligated, so that blood is cut off altogether from the liver, and the output of urea is definitely increased under these circumstances by the subcutaneous administration of amino-acids. We can hardly doubt therefore that other tissues besides the liver possess the power of manufacturing urea, although the size and functional activity of the liver enable it to play a predominant role in this, as in other chemical phenomena in which it plays a part.

The question which next arises is that of the chemical origin or precursor of urea. A direct origin from **Arginine** is immediately suggested by mere inspection of the structural formula of this amino-acid:

$$NH = C\begin{cases} NH_2 \\ NH.CH_2.CH_2.CH_2CH(NH_2)COOH \end{cases}$$

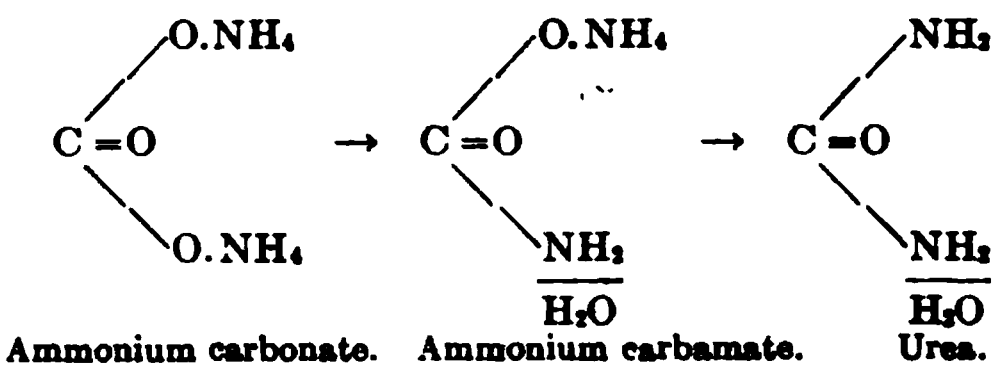
and since the discovery by Kossel and Dakin of the existence of an enzyme, **Arginase** in aqueous extracts of the liver, spleen, thymus and intestinal mucosa which directly splits arginine with the production of urea, and **Ornithine**:



there can be no doubt that a proportion of the urea output originates in this manner. It can only be a small proportion, however, since urea forms over eighty per cent. of the total nitrogen output and only a very small percentage of the nitrogen intake is in the form of arginine radicals.

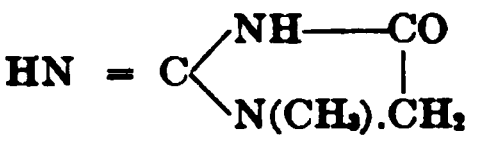
The origin of the greater part of the urea output is undoubtedly to be traced to **Ammonia** formed by deaminization from the various amino-acids. We have seen that the decrease of urea output which accompanies interference with the liver-functions also results in a corresponding increase of the ammonia output in the urine, and this fact in itself would point to ammonia as a precursor of urea. It can, however, be directly shown that when ammonia in the form of **Ammonium Carbonate** is supplied to the liver, it is transformed therein into urea. Thus Nencki and Pawlow have shown that the percentage of ammonia contained in the blood from the portal vein is considerably higher than it is in the blood from the hepatic vein, showing that the ammonia is retained by the liver as the portal blood passes through it. Furthermore, when ammonium carbonate is administered to animals it appears in the urine as urea, and, finally, von Schroeder perfused the isolated liver of the dog with ammonium carbonate and obtained, not only the retention of ammonia observed by Nencki and Pawlow, but also an actual replacement of the perfused ammonium carbonate in part by urea. **Ammonium Formate** was similarly transformed. The conversion of ammonium salts into urea by the tissues of the liver has therefore been confirmed in a variety of ways.

Urea is the diamide of carbonic acid and may be derived from carbonic acid by the successive introduction of amino-groups, an intermediate stage of the process being the formation of **Carbamic Acid**:

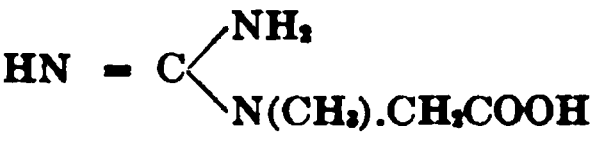


Now it has been shown by Macleod and Haskins that there is an equilibrium in aqueous solutions between ammonium carbonate and **Ammonium Carbamate**, so that if the ammonium carbamate is removed by transformation into urea a continuous renewal of the ammonium carbamate is to be expected, and consequently a quantitative conversion of the ammonium carbonate into urea. The formation of ammonium carbamate as an intermediate product in the synthesis of urea in the body is shown by the fact that if alkalies be administered to animals in considerable quantity carbamates appear in abundance in the urine. A direct conversion of ammonium carbamate into urea has been accomplished by Drechsel by simply passing an alternating current through its solution, *i. e.*, by alternate oxidation and reduction which is, of course, equivalent to dehydration. We may infer, summing up the results of these various investigations, that ammonia, derived from amino-acids by the process of deaminization, is converted by union with carbon dioxide into ammonium carbonate, which spontaneously undergoes partial transformation into ammonium carbamate. The latter substance is converted by alternate oxidation and reduction in the liver into urea which is subsequently expelled from the body by the kidneys. In **Acidosis**, whether induced by disordered metabolism or by the ingestion of acids in excess, this process is impeded and the ammonia is utilized in part to neutralize the excess of acids in the blood and tissues. The output of **Ammonia** in the urine, therefore, rises in acidosis and is, in fact, a most valuable means of detecting and estimating the severity of that condition.

Next to urea, but as a rule far inferior to it in amount, the most abundant nitrogenous constituent of the urine is **Creatinine**:



this substance may be regarded as an anhydride of **Creatine**, or methyl guanidine acetic acid:



which, it will be remembered, is an abundant constituent of muscular tissues.

The daily output of creatinine in man is from 1.0 to 1.7 grams or from four to six per cent. of the total nitrogenous excretion. Our views regarding the probable origin of creatinine have undergone very important modifications in recent years, thanks to the fundamental investigations of Folin, Van Hoogenhuyze and Verploegh, and Mellanby. It was formerly assumed without any doubt that the source of the creatinine in the urine was the creatine in the muscular tissues. This must now be considered to have become uncertain, and in any

case we have come to attach a very fundamental significance to the creatinine excretion in the urine.

It was first pointed out by Folin that with varying nitrogenous intakes the behavior of the creatinine output is fundamentally different from that of the output of urea. The latter rises and falls almost in direct proportionality to the quantity of protein in the food. The creatinine output, on the contrary, remains almost unaltered whether the protein content of the diet be high or low. The creatinine output is not, therefore, derived from the diet. Thus, for example, Folin compared the urea and creatinine excretion on a high protein diet and a low protein diet, with the following very striking results:

	High protein diet.	Low protein diet.
Volume of urine	1170. c.c.	385. c.c.
Total nitrogen	16.80 grams	3.6 grams
Urea-nitrogen	14.70 "	2.2 "
Creatinine-nitrogen	0.58 "	0.6 "

The urea output, it will be seen, fell on the low protein diet to one-sixth of that obtained on the high protein diet. The creatinine output, on the contrary, remained almost unaltered.

The statement that the creatinine which is excreted in the urine is not derived directly from the foodstuffs must be qualified to this extent, that if creatinine be contained preformed in the diet, the greater part of it is excreted in the urine unaltered within twenty-four hours. On the other hand, if creatine be administered with the food it does not appear in the urine either in the form of creatine or creatinine. In fact it usually appears to be excreted by some other channel or else retained by the body, for Folin in many instances administered creatine without causing any increase even in the total nitrogen of the urine. It has been suggested by Mellanby that bacteria in the intestine decompose the creatine and retain it in their tissues. However this may be, these observations render it certain that the creatine which is contained preformed in a meat-diet is not the source of the creatinine in the urine.

Since the output of creatinine is so extraordinarily independent of fluctuations in the diet, Folin regards it as originating in the **Endogenous Metabolism** of the tissues themselves, while a great part of the urea arises from the destruction by deaminization of amino-acids which have never become part of the living protoplasm of the body, and therefore represents a product of **Exogenous Metabolism.** The exogenous metabolism rises and falls with the intake of foodstuffs, but the endogenous metabolism persists practically unchanged under a variety of nutritional conditions. It represents the "wear and tear" or irreversible spontaneous decomposition of the tissues.

It is questionable, however, whether the creatinine output represents the endogenous metabolism of the whole body or whether it does not, on the contrary, arise from the endogenous metabolism of the muscular

tissues only. The daily output of creatinine, although so constant in a given individual, varies in different individuals with the weight, and more especially with the degree of muscular development. Obese persons, notwithstanding their high body-weight, have a low creatinine output, while comparatively lean persons, who by virtue of muscular development have a like weight, exhibit a high creatinine output. It is true that muscular work on a normal diet does not increase the creatinine output, but then we have seen that on a normal mixed diet the muscles do not derive their energy from the metabolism of their own substance (protein) but from the oxidation of carbohydrates. When, however, muscular work is performed during starvation, the creatinine output is definitely increased. In other words the actual destruction of muscular tissue results in an increase of creatinine excretion.

It appears very probable that the normal products of the disintegration of tissue-protein are similar to or identical with the substrates out of which tissue-protein is synthesized, namely, the amino-acids, for we have seen that the process of tissue-synthesis is a balanced reaction which is retarded by its products, and this can only be true if the products of the synthesis break down, in the first place, into the substances which form the substrates of the forward reaction. The amino-acids which are thus set free are cast into the general stock of circulatory and storage amino-acids, undergo their share of exogenous metabolism or deaminization, and participate with the ingested amino-acids arising from the foodstuffs in determining the **Nutrient-level** of the tissue-fluids. If the nutrient-level falls, as in starvation, the amino-acids of tissue origin form a large proportion of the whole mass of circulating amino-acids, and their deaminization results in a continual drainage which, in turn, results in a steady loss of tissue-substance. There must, in fact, be an endogenous or tissue-source of urea, for otherwise urea excretion would ultimately fall to zero in starvation, which it never does. In fact, even in starvation the urea output still exceeds very decidedly the creatinine output. On the other hand, if the tissues must use their own substance for the performance of external work, at any rate in muscular tissues, the breakdown of the protein or of amino-acids resulting therefrom takes another course, with the production of creatinine. The effect of this must be to initiate a process analogous to repair or **Regeneration** by the resynthesis of the lost tissue-proteins from amino-acids.

Creatine is not a normal constituent of the urine of adult men and, as has been stated above, the ingestion of creatine leads to no increase in the creatinine output, nor does it lead to the appearance of any creatine in the urine. In the urine of women, on the contrary, creatine is found during menstruation and after delivery, and the ingestion of creatine leads to the appearance of a small proportion of the creatine in the urine. In the urine of children creatine is a regular constituent. According to Krause it disappears from the urine of boys at about five

or six years of age, but persists in the urine of girls until puberty. The ingestion of creatine in children is also followed by an increase in the creatine output in the urine. The adult has therefore acquired a power of destroying or utilizing creatine which is imperfect in women and only slightly developed in young children.

Apart from the question of the nature of the tissues in which creatine and creatinine originate we have to consider the problem of the chemical precursors or parent-substances from which they originate. A very obvious possibility is that they may arise from **Arginine.**

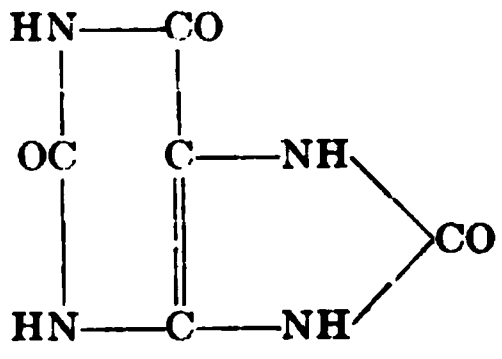
$$HN = C\begin{matrix}\diagup NH_2 \\ \diagdown NH.CH_2.CH_2.CH_2.CHNH_2.COOH\end{matrix}$$

by breaking the hydrocarbon-chain and methylation of one of the nitrogens in the guanidine nucleus. It has been stated that creatine may arise from proteins in the autolytic decomposition of tissues in the absence of bacteria but no other evidence of its formation from arginine has yet been adduced.

Creatinine is a reducing agent and decolorizes cupric hydroxide in alkaline solutions, but does not precipitate cuprous oxide as the reducing sugars do. It is precipitated by **Picric Acid**, but if treated with picric acid in alkaline solutions it yields a red coloration which turns yellow upon the addition of acids (**Jaffe's Reaction**). If an alkaline solution is treated with **Sodium Nitroprusside** the mixture turns ruby red (**Weyl's Reaction**) and then yellow. If this yellow solution is treated with excess of acetic acid and boiled, it becomes first green and then blue (**Salkowski's Reaction**). Jaffe's reaction is utilized by Folin for the colorimetric estimation of creatinine in urine. Creatine is estimated by converting it into creatinine by boiling with dilute acid and then reëstimating the creatinine.

Uric Acid is an exceedingly important constituent of the urine, since it represents, in man, the end-product of the purine metabolism. The average output per day on a mixed diet is 0.7 grams, and the ratio of uric acid to urea varies between 1 : 50 and 1 : 70.

Uric acid is derived from the **Purine Bases** by oxidation; it is 2, 6, 8, trioxypurine:

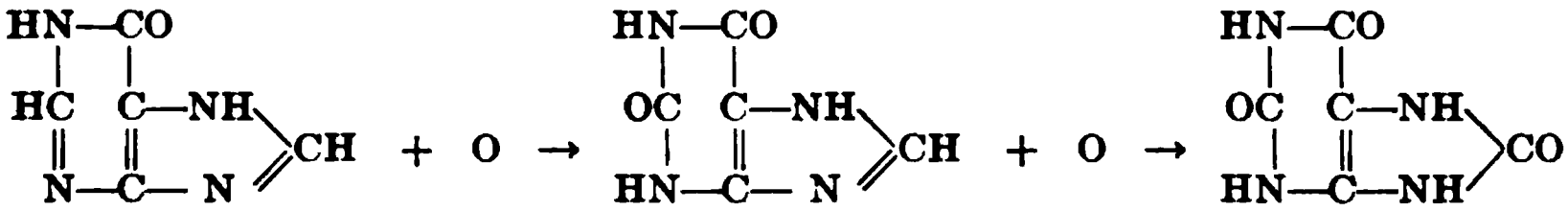


It may be prepared synthetically from urea and glycocoll. On heating in sealed tubes with hydrochloric acid, glycocoll, carbon dioxide and ammonia are produced. It is capable of acting as a weak acid and forms two series of salts, the **Monourates**, containing one, and

the **Diurates**, containing two molecules of base. The so-called quadriurates are non-existent.

Uric acid yields a variety of characteristic color reactions, among which the **Murexide Test**, already described in connection with the purine bases, must be included. Uric acid is a reducing agent and reduces an alkaline cupric hydroxide solution; the quantity of uric acid which is present in urine is, however, insufficient to produce an appreciable precipitation of cuprous oxide. If a drop of uric acid dissolved in sodium carbonate be placed upon a filter-paper moistened with silver nitrate solution, reduction occurs with the production of a yellow or brown spot (**Schiff's Reaction**). If a weak alkaline solution of uric acid in water is treated with a soluble zinc salt a white precipitate is produced which gradually turns blue if exposed to light and air, or immediately, if treated with sodium persulphate (**Ganassini's Reaction**). With a certain mixture of phosphoric and phosphotungstic acids uric acid yields a blue coloration (**Folin and Macallum's Reaction**), the origin of which is unknown.

The elimination of uric acid is definitely increased by a diet which contains excess of purines or of **Nucleic Acids**. This is due to the fact that the adenine and guanine, split off from the nucleic acids, are transformed in the tissues into **Hypoxanthine** and **Xanthine**, by the deaminizing enzymes adenase and guanase. The hypoxanthine is subsequently converted into xanthine and the xanthine into uric acid by a specific oxidizing enxyme which is found in a variety of animal tissues, and is designated **Xanthine-oxidase**:



Hypoxanthine. **Xanthine.** **Uric acid.**

Nevertheless, the elimination of uric acid continues on a purine or nuclein-free diet. In a series of experiments on himself and others, Folin was able to reduce the daily elimination to 0.3 grams on a diet of cream and starch, but this minimum could not be reduced. Evidently, therefore, there is, as in the case of amino-acids and other foodstuffs, an **Endogenous Metabolism** of purines as contrasted with an **Exogenous Metabolism.** That the endogenous metabolism represents the actual breaking down of tissues is shown by the fact that if destruction of tissue is remarkably augmented, as in pneumonia, leukemia, or in severe burns, the uric acid excretion rises decisively.

There is no evidence that mammalian tissues can synthesize uric acid from any other source than purines. It is true that the elimination of uric acid, and of purine bases also, is increased by an increase in the dietary intake, but this is true whether the increase be nitrogenous or non-nitrogenous, and it follows very rapidly upon the intake of food.

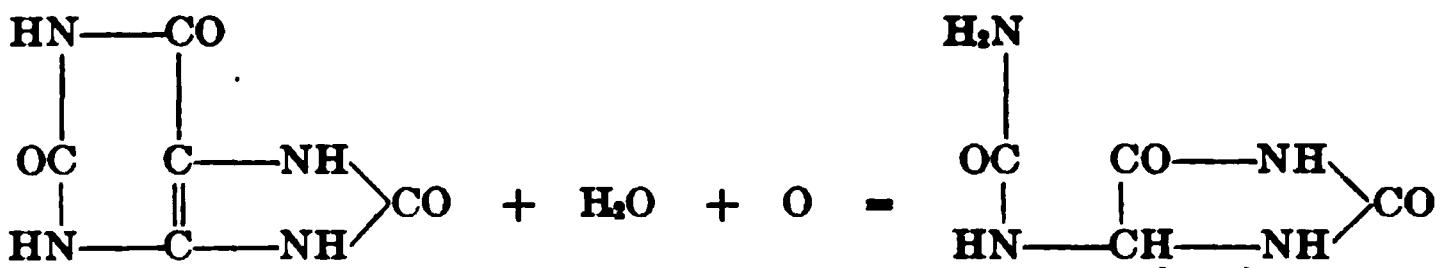
Thus Hopkins and Hope, after fasting for six hours, consumed a meal of bread and potatoes, pratically purine-free, with the following results:

Time	Urea, grams.	Uric acid, milligrams.
10 to 11	1.07	26
11 to 12	1.13	27
12 to 1	1.07	24
1 to 2 (meal at 1.30)	0.64	21
2 to 3	1.12	22
3 to 4	1.16	38
4 to 5	0.84	40
5 to 6	1.16	56
6 to 7	1.20	39
7 to 8	1.37	30
8 to 9	1.47	33
9 to 10	1.33	24
10 to 11	1.33	23

Thus a slight rise in the urea output occurred about six hours after the ingestion of the food, and continued for some time, but a sharp rise in the uric acid output occurred within two hours, and the excretion fell tc nearly the normal value again before the urea excretion began to rise. It is not known where this uric acid originates, but it would appear to be manifestly connected with the activities of the alimentary canal, and to be endogenous in origin. It is for this reason that the uric acid and purine output is greater during the day than it is at night.

In birds and reptiles the relationships are quite different. These possess the power of synthesizing uric acid, most probably from **Ammonia** and **Lactic Acid**, since, if the liver be extirpated in birds, the place of the uric acid in the excreta is taken by ammonia, and large amounts of lactic acid are excreted concurrently. An increase of uric acid elimination in birds follows the administration of lactic acid and other hydroxy-acids and dibasic acids of the aliphatic series. This power is, however, lacking in the mammalia.

In the majority of mammals, uric acid is not the end-product of the purine metabolism, but undergoes in part or almost wholly, transformation into **Allantoin** which is excreted in the urine:



This transformation, which is known as **Uricolysis**, is brought about by an oxidizing enzyme, **Uricase**, which occurs in tissue-extracts prepared from the liver, kidney and other organs. It trantforms uric acid almost quantitatively into allantoin. It is probable, however, that the destruction of uric acid does not stop at this stage but proceeds further and, ultimately, to the formation of urea and other products. Thus Ascoli

and Izar have shown that if an extract of liver which has completely destroyed a given sample of uric acid in the presence of oxygen be excluded from oxygen, the uric acid is gradually reformed. This is what one would expect if we had here to deal with a reversible oxidation. The curious feature of their results is, however, that the addition of allantoin had no effect upon the production of uric acid, appearing to indicate that the production of allantoin was not an intermediate step in the resynthesis.

The power of uricolysis is absent from the tissues of man and the chimpanzee—a fact which would have gladdened the heart of Huxley, could he but have known it. All other mammals, so far as we know, contain uricase in their tissues. The following results, cited after Hunter and Givens, show the relative proportions of uric acid and allantoin in the urine of various mammals. The "**Uricolytic Index**" is the proportion, expressed as a percentage of uric acid, which has been converted by the animal into allantoin.

Orders and species.	Total purine nitrogen, gms.	Percentage of purine-allantoin-nitrogen.			Uricolytic index.
		Allantoin.	Uric acid.	Bases.	
Marsupialia:					
Opossum	0.04	76.0	19.0	6.0	79
Rodentia:					
Rabbit	..	..	..	..	95
Guinea-pig	..	91.0	6.0	3.0	94
Rat	..	93.7	3.7	2.7	96
Ungulata:					
Sheep	0.2 to 0.6	64.0	16.0	20.0	80
Goat	1.0	81.0	7.0	12.0	92
Cow	8.0	92.1	7.3	0.7	93
Horse	1.6	88.0	12.0	0.5	88
Pig.	0.3	92.3	1.8	5.8	98
Carnivora:					
Raccoon	..	92.6	5.4	2.0	95
Badger	0.25	96.9	1.9	1.2	98
Dog	0.1 to 0.3	97.1	1.9	1.3	98
Coyote	0.15	95.6	2.6	1.8	97
Primates:					
Monkey	0.045	66.0	8.0	26.0	89
Chimpanzee	..	..	..	..	0
Man	0.2	2.0	90.0	8.0	2

Allantoin has been isolated by Hunter from the blood of the ox, pig, horse and sheep, but could not be detected in the blood of man.

It is not by any means certain, however, notwithstanding the inability to convert uric acid into allantoin, that the tissues of man cannot destroy uric acid in some other manner. Thus, Taylor and Rose fed a human subject for three days on a diet very low in purines, namely milk, eggs, starch, and sugar. For three days following, a part of the protein, namely three grams per day out of a total of ten was given in the form of "sweetbread" nitrogen (sheep's pancreas). For four days succeeding this twice as much "sweetbread" nitrogen was given, namely six out of ten grams, and this was succeeded by a

period of four additional days on a purine-free diet. The following were the results obtained:

	1st period, purine-free diet.	2d period.	3d period.	4th period, purine-free diet.
Total urinary N . . .	8.9	8.7	9.1	8.80
Urea N + NH_3 . . .	7.3	7.1	7.1	7.05
Creatinine N . . .	0.58	0.55	0.56	0.47
Purine N (total) . .	0.11	0.17	0.26	0.10
Uric acid	0.09	0.14	0.24	0.07
Undetermined N . .	0.91	0.88	1.18	1.18

The intake of purine nitrogen in the second period was 0.17 and in the third 0.34 grams per day, so that the increased output only accounted for one-half of the intake. The purine was not simply stored, to be excreted later, for as soon as the purine-rich diet ceased the excretion fell to the figure previously obtained on a purine-free diet. The only alternatives that remain are either that part of the purine was never absorbed from the intestine or else that the tissues of the subject destroyed the purines in some manner which did not result in the formation of uric acid or allantoin. We may recall the observations of Ascoli and Izar, cited above, which tend also to the conclusion that there are means of destroying uric acid in the tissues which do not involve the production of allantoin as an intermediate stage.

In persons afflicted with **Gout** deposits of uric acid form in various tissues and particularly in the joints. The origin of these deposits has been the subject of much investigation. There is a definite increase in the uric-acid content of the blood in such persons, although the uric-acid output in the urine is not above the normal. Evidently, therefore, the kidneys are functioning abnormally and in such a way as to constitute a barrier to the excretion of uric acid. The limiting concentration in the blood at which transmittal through the renal epithelium begins is raised, and hence the uric acid, dammed back in the blood, accumulates therein. This alone, however, is not a sufficient cause of gout, for uricemia occurs also in nephritis, and in lead poisoning, without the production of gouty deposits. It has been suggested that the solubility of uric acid in the blood is diminished in gouty persons, but no positive evidence of this has been advanced. The origin of the tendency of uric-acid deposits to form in the joints when they do occur at all is, however, rendered clear by the fact upon which emphasis is laid by Taylor, that **Cartilage**, possibly owing to its high content of sodium salts, diminishes the solubility of sodium urate in water, so that deposits are precipitated upon it from saturated solutions.

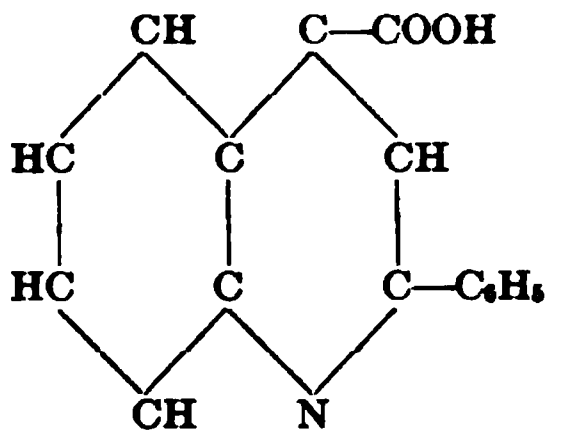
The solubilities of the monourates of potassium, sodium and ammonium at 37° C. in water have been determined by Gudzent as follows:

Salt of uric acid.	Solubility in grams per liter.
Potassium	2.7002
Sodium	1.5043
Ammonium	0.7413

The solubility of sodium urate in blood is, however, no less than three times its solubility in water (Taylor). This is not due to the formation of diurates, since at the reaction of the blood diurates cannot exist. The nature of the factor which so greatly increases the solubility of uric acid is unknown.

It was formerly considered possible to remove uric acid from the body by administering **Alkalies**, the assumption being that the greater alkalinity of the blood resulted in the formation of the more soluble diurates. We now know that the alkalinity of the blood is only increased to an almost imperceptible extent by this means and that the maximum alkalinity attainable would not suffice to form diurates, or indeed to influence perceptibly the solubility of uric acid. Nevertheless, the administration of certain alkalies may be assumed to facilitate the solution of uric acid by the formation of a certain proportion of the more soluble potassium salt, or of the **Lithium Urate** which is the most soluble salt of uric acid.

The most remarkable effect upon the elimination of uric acid is, however, that of phenylquinoline-carbonic acid or **Atophan**:



The administration of this substance and of other quinoline-carbonic acid derivatives has been shown by Nicolaier to increase the amount of uric acid excreted by the kidneys to an extraordinary extent, even to twice or three times the normal amount. No other physiological effects are noted and no other constituent of the urine is altered in amount. The increased elimination occurs on a purine-free diet and has been shown by Folin and Lyman to be accompanied by a fall in the uric acid content of the blood. In other words the hyperexcretion of uric acid is due to the increased permeability of the kidneys for this substance, just as the glycosuria following phloridzin administration is due to increased permeability of the kidneys for glucose. The hyperexcretion does not persist if the administration be continued, the daily output sinking within a few days to only slightly above the normal level, probably because the available supply of urates in the blood and tissue-fluids has become exhausted. There is, however, a continuous slight hyperexcretion throughout a prolonged period of administration, and when nuclear tissues are administered in the diet a greater proportion of uric acid is excreted in consequence than is usually the case. The formation of uric acid from the nucleic acids is thus facilitated by atophan, but this effect is probably only a secondary

one, depending upon the reduction of the concentration of the urates in the tissue-fluids, and the tendency of the tissue-enzymes to spontaneously reëstablish the normal equilibrium between the blood and the tissues.

The only amino-acid which normally occurs in urine is **Glycocoll**, or amino-acetic acid, which, in very small amounts, appears to be a constant constituent. If, however, an excess of leucine or alanine be introduced into the circulation they will appear in the urine. It would appear that, normally, deaminization and utilization are too rapid to permit of the accumulation of amino-acids in the blood in sufficient amount to cause elimination by the kidneys. If, however, the rate of deaminization be slowed, as, for instance, in degenerative changes of the liver induced by chloroform-necrosis or phosphorus-poisoning, then a variety of amino-acids may appear in the urine. It is also stated by Loewy that the amino-acid content of the urine is increased at high altitudes.

When the urea, creatinine, uric acid and glycocoll of the urine are added together, there is always a considerable remainder of nitrogenous excretion. Part of this arises from the sulphur-containing and conjugated excreta which are about to be described, part is stated by Abderhalden and Pregl to be present in the form of **Polypeptides** which yield glycocoll, leucine, alanine, glutamic acid and phenylalanine on hydrolysis. When all the nitrogen in hitherto defined substances is summed up, however, there is still a small remainder which, although it arises from substances excreted in small amount, may nevertheless be of physiological importance. It is derived in part from exogenous and in part from endogenous metabolism.

CONJUGATED EXCRETA.

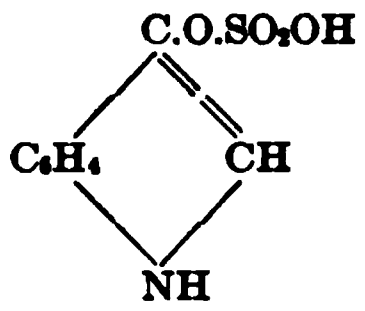
A variety of substances occur in the urine which arise from the union of a genuine excretory product with another molecule which serves as a vehicle to accomplish its elimination. Such excreta are, for example, the **Conjugated Glucuronic Acids** which are normally present in the urine in small amounts and are greatly augmented by the ingestion of certain poisons, of which a partial list has been given in a previous chapter (Chapter III). The function of the glucuronic acid moiety of the molecule appears to be in the main to render harmless the associated substance which is usually of a toxic character. Only definite classes of toxic substances are eliminated in this manner, however.

The **Glucuronates** which normally occur in the urine are in the main the phenyl, indoxyl and skatoxyl glucuronates, the latter two in very small amounts, The phenol, indoxyl and skatoxyl radicals are derived, it is believed, mainly from putrefactive decomposition of aromatic amino-acids, particularly tyrosine and tryptophane, by the intestinal bacteria. These substances are in themselves very toxic, but their

conjugates with glucuronic acid are harmless. Upon boiling with dilute acids or occasionally even on allowing urine to stand, they decompose, setting free glucuronic acid and the associated radical of the conjugate.

The origin of the glucuronic acid in urine is unknown. The most natural assumption is to suppose that the toxic substances which are eliminated in this way combine in the body with glucose, and that the oxidation of glucose is by this so hindered, that it only proceeds as far as the conversion of the primary alcohol-group into a carboxyl-group. Certainly the phenyl-glucuronic acid is a compound of the glucoside type, *i. e.*, the phenyl radical is attached to the glucuronic acid by the aldehyde-group. On the other hand if camphor be administered in large amounts to phloridzinized dogs, although the excretion of glucuronates is very greatly increased thereby, the excretion of glucose is either not diminished at all or only slightly diminished, a fact which would appear to indicate some other source than glucose for the glucuronic acid.

A very important excretory conjugate is the conjugated sulphuric acid, indoxyl-sulphuric acid or **Indican**:



which yields **Indigo** when treated with oxidizing agents. This substance arises by conjugation of indoxyl with sulphuric acid and is the form in which the greater part of the indoxyl output is present in the urine. The indoxyl output varies with the extent of putrefactive processes in the intestine. Any measure of **Intestinal Stasis**, such as that induced by tying off a loop of small intestine, results in an increase of the indican output. The subcutaneous injection of indol leads to an increased output of indican, while the administration of an excess of **Tryptophane** in this way does not. Evidently the tissues do not decompose tryptophane in such a way as to liberate indole, while the intestinal bacteria, like the majority of putrefactive bacteria, generate a large proportion of indole from tryptophane, which, after absorption is oxidized to indoxyl and then excreted in the form indicated above. It must be remembered that the indican output, although generally running parallel with the degree of intestinal stasis or putrefaction, is not a reliable measure of intestinal putrefaction when taken by itself, for the output depends, not solely upon putrefaction, but also upon the proportion of tryptophane which is contained in the proteins of the diet. Thus, if a large part of the protein intake be supplied by **Gelatin**, which contains no tryptophane, the indican output becomes very small although putrefactive processes may not be diminished in

the slightest degree. Then, again, even upon a standard diet, the output of indican may be expected to vary greatly with the type of infecting organisms in the intestine. Thus Herter has shown that *Bacillus coli communis* produces indole but only traces of skatole, which is the methyl derivative of indole, while certain anaërobic putrefactive bacteria produce skatole, in preference to indole, from tryptophane. Skatol does not appear to be normally excreted in the urine, at least in the form of a conjugated sulphuric acid.

Phenol-sulphuric Acid and **Cresol-sulphuric Acid** are constant constituents of urine, and, as in the case of indican, the output is obviously derived from the products of intestinal putrefaction. It is probable that these substances, of which the total excretion may amount to fifty milligrams per day, originate from the putrefactive decomposition of **Tyrosine** and **Phenylalanine.**

In general it may be said that while aliphatic alcohols, terpenes and many phenols are excreted in the urine in conjugation with glucuronic acid, the greater part of the phenols and polyphenols are excreted in conjugation with sulphuric acid. Yet a third vehicle of excretion is that afforded by conjugation with **Glycocoll**, or amino-acetic acid. Thus **Benzoic Acid**, appears in the urine after administration in the form of the conjugated **Hippuric Acid**:

$$\underset{\text{Benzoic acid.}}{C_6H_5COOH} + \underset{\text{Glycocoll.}}{CH_2NH_2COOH} = \underset{\text{Hippuric acid.}}{C_6H_5COHNCH_2COOH} + H_2O$$

Hippuric acid is a very abundant constituent of the urine in Herbivora, comparatively scanty in the urine of Carnivora, and intermediate in amount in the urine of partially herbivorous animals like ourselves. The daily excretion in man, subsisting upon a normal mixed diet, is about 0.7 grams, but after eating quantities of vegetables or fruits it may rise as high as 2 grams.

The synthesis of hippuric acid from benzoic acid and glycocoll is accomplished within the tissues of the kidneys themselves. This, in fact, was the first synthetic process which was definitely shown to take place in animal tissues (by Schmiedeberg and Bunge) and also the first to be performed by admixture of the components of the reaction with macerated tissue. It is not improbable, however, that some measure of hippuric acid synthesis may also occur in other organs.

When large amounts of benzoic acid are administered to animals the elimination of glycocoll is far in excess of the glycocoll which could be obtained by simple hydrolysis of the protein. Thus McCollum and Hoagland brought a pig into a condition of minimal nitrogen metabolism by administering a diet of starch containing 75 calories per kilogram body-weight. To this diet was then added varying amounts of benzoic acid, and finally hydrochloric acid and benzoic acid were given together. The total nitrogenous output and its partition among the various nitrogenous fractions in the urine were determined in the different periods of the experiment with the following results:

Period.	No. of days.	Food.	Total N.	Urea N.	NH_3 N.	Creatinine N.	Hippuric acid + other N.
I	12	Starch, 75 cal. per kilo + alkali salts	2.56	1.43	0.21	0.488	0.424
II	4	Same + 4 g. benzoic acid	2.63	1.29	0.21	0.456	0.681
III	7	Same + 10 g. benzoic acid	2.23	0.58	0.22	0.484	0.948
IV	5	Same + 16 g. benzoic acid	2.86	0.55	0.38	0.437	1.492
V	5	Same + 16 g. benzoic acid + 10 g. of 25 per cent. HCl	4.03	0.54	1.44	0.424	1.632

It will be seen that despite the great increase of hippuric acid excretion induced by these large dosages of benzoic acid the total daily nitrogen elimination was unaffected. Evidently body-protein was not attacked to provide the glycocoll needful for the synthesis of the hippuric acid. The glycocoll was evidently derived at the expense of the urea-fraction, and the endogenous catabolism, in so far as it is represented by the creatinine output, remains unaffected. On the other hand the acidosis induced by hydrochloric acid resulted in a large increase of the total nitrogen output, the chief part of the increase being **Ammonia** which performs the protective function of neutralizing a part of the excess of acid. The urea and creatinine output were alike unaffected by the administration of the acid.

The glycocoll moiety of hippuric acid must therefore be traced to the same origin as urea, and this, it will be remembered, is the amino-acids of the tissue-fluids. No less than thirty-five per cent. of the nitrogen of the food may be excreted as hippuric acid, and no protein contains this percentage of glycocoll. It is evident that glycocoll may be synthesized from other amino-acids. It might be imagined that the benzoic acid unites with other amino-acids which thereafter undergo partial oxidation until only the residue of glycocoll is left. Injection of such compounds synthetically prepared, however, leads to no increase in the hippuric acid output. It seems probable, therefore, that glycocoll may form a normal disintegration-product of many amino-acids, that under ordinary circumstances it is finally deaminized, but that when toxic substances that will pair with it, namely aromatic acids, are present in the tissue-fluids, deamination is prevented by the conjugation.

The power of the tissues to synthesize glycocoll is of very great importance, since it not only enables the body to protect itself against such poisons as benzoic acid, but also enables suckling animals to synthesize their tissue-proteins from a protein which is totally lacking in glycocoll, namely the casein of milk.

When the administration of benzoic acid is pushed beyond the limit of the glycocoll available from the proteins of the diet the protective mechanism breaks down and free benzoic acid appears in the urine. Under no circumstances, it appears, are tissue-proteins attacked

for this purpose nor are the proteins of the blood broken down to furnish glycocoll, for the ratio of albumins (containing no glycocoll) to globulins in the blood-serum remains unaltered by benzoic-acid administration.

In the metabolism of birds, **Ornithine**, or diaminovaleric acid plays

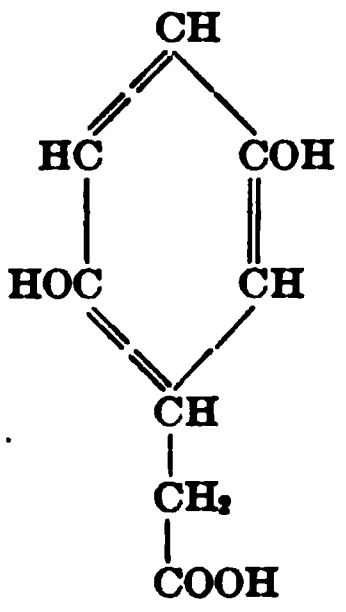
$$CH_2(NH_2)CH_2.CH_2.CH(NH_2)COOH$$

the part which is taken by glycocoll in the metabolism of mammals, or, at all events, to the extent of being the substance utilized to detoxicate and eliminate benzoic acid. The conjugated acid which appears in the urine of birds when benzoic acid is administered to them is **Ornithuric Acid**, which splits into benzoic acid and ornithine when it is hydrolyzed.

AROMATIC OXYACIDS.

The putrefaction of proteins in the intestine results in the formation of **Paraoxyphenylacetic Acid** and **Paraoxyphenylpropionic Acid** as intermediate stages in the decomposition of tyrosine, and they pass in small amounts unchanged into the urine.

It has been observed, from the middle ages, that human urine in certain very rare instances may regularly darken on exposure to air and ultimately turn black. The individuals exhibiting this peculiarity, which is designated **Alcaptonuria**, are very rare, and yet the condition constitutes a definite peculiarity of metabolism which has often been described, and has been very carefully investigated. The darkening is due to the spontaneous oxidation of dioxyphenyl acetic acid or **Homogentisic Acid**:



which is a constituent of the urine of these persons. The individuals who display this peculiarity do not appear to suffer any inconvenience from it, and cases only reach the physician through the alarm created by the extraordinary appearance of the urine after standing, or by failure to secure an insurance-policy, for dioxyphenyl acetic acid is a reducing-substance and may be reported by a physician who is unfamiliar with the typical indications of the disease, as glycosuria. The

reduction of cupric hydroxide solution by the urine of an alcaptonuric individual is, however, accompanied by darkening or even blackening of the fluid, so that no confusion of diagnosis should be possible even on superficial observation.

The homogentisic acid in alcaptonuria arises from the tyrosine and phenylalanine radicals in the proteins of the food. If the diet contains little tyrosine or phenylalanine the output sinks, if much it rises. The administration of tyrosine or phenylalanine by mouth, or of glycyltyrosine hypodermically, leads to quantitative excretion of the aromatic nucleus in the form of homogentisic acid. The elimination continues, although it is reduced, in starvation, and this, together with the fact that it may be enhanced by subcutaneous or intravenous administration of the parent acids, shows that the homogentisic acid is not derived from intestinal cleavage or putrefaction.

Evidently the alcaptonuric is unable to complete the oxidation of the aromatic nuclei of **Tyrosine** and **Phenylalanine**, just as the Diabetic is unable to complete the oxidation of β-oxybutyric acid. Curiously enough, however, as Garrod and Neubauer have shown, tryptophane is normally utilized by persons who display alcaptonuria.

According to Garrod there is but one degree of alcaptonuria and that is complete. Either the excretion of homogentisic acid amounts to several grams a day or it is absent from the urine, and usually the condition is present from earliest childhood. It is evidently the exogenous metabolism only of tyrosine and phenylalanine which is affected for no defect of development or loss of weight in the adult occurs such as we would expect to happen, were tissue-protein destroyed to produce the homogentisic acid. It is the circulating amino-acids, which normally undergo complete combustion after deaminization, which are the source of this substance.

It is probable that homogentisic acid represents a normal intermediate product in the oxidation of the oxyphenyl-oxypropionic acid which results from the deaminization of tyrosine. The curious feature of the transformation, however, resides in the fact that whereas tyrosine has only one hydroxyl-group in the benzene nucleus and that in the para position, homogentisic acid has two, one in the ortho and the other in the meta position. It is found, however, that this is the only class of dioxyphenols which is oxidized by normal persons, other dioxyphenols being excreted in the form of conjugates in the urine. The alcaptonuric therefore differs from the normal person in that his inability to oxidize diphenols extends to the single class which normal individuals can oxidize, namely those in which the hydroxyl-groups occupy the ortho and meta positions relatively to the side-group.

WASTE-PRODUCTS OF THE SULPHUR METABOLISM.

The waste-products of the sulphur metabolism are of three types, namely **Inorganic Sulphates, Ethereal** or **Conjugated Sulphates** and the

Neutral Sulphur compounds in which the sulphur is not present as a sulphuric acid radical.

These three fractions have been found by Folin to vary in a characteristic manner with the abundance of proteins in the diet. On high and low protein diets respectively the following daily output of the various sulphur-containing excreta was observed:

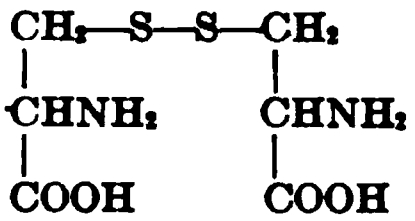
	Protein-rich diet.		Protein-poor diet.	
Volume of urine	1170 c.c.		385 c.c.	
Total nitrogen	16.80 gm.		3.60 gm.	
Total sulphur (SO_3)	3.64 "		0.76 "	
Inorganic SO_3	3.27 "	(90.0 per cent.)	0.46 "	(60.5 per cent.)
Ethereal SO_3	0.19 "	(5.2 per cent.)	0.10 "	(13.2 per cent.)
Neutral SO_3	0.18 "	(4.8 per cent.)	0.20 "	(26.3 per cent.)

It will be observed that a reduction of the total sulphur output to one-fifth, reduced the output of inorganic sulphates to one-seventh, and of ethereal sulphates to one-half, while the output of neutral sulphur remains unaltered. Folin draws an analogy between the neutral sulphur output and the creatinine output among the nitrogenous excreta, and regards the neutral sulphur as originating from the degeneration of tissue-protein, the **Endogenous Metabolism**, while the inorganic sulphates represent the extent of **Exogenous Metabolism** or the destruction of circulating amino-acids which have not become constituents of living tissue.

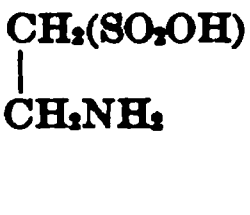
The ethereal sulphates, representing conjugated phenols, indican and so forth, have usually been regarded as indicative of the extent of **Intestinal Putrefaction**. The relatively slight degree to which they are reduced by a reduction of protein intake to one-half is adduced by Folin as an indication that they may possibly arise from the endogenous metabolism of tissues. It is to be noted, however, as Hopkins has pointed out, that we have no right to assume that **Intestinal Putrefactions** are reduced proportionately to the reduction of the protein intake. On the contrary, the proportion of the protein intake which reaches the lower intestine without absorption is as a rule very small, unless it chances to be a form of protein which is indigestible, such as raw egg-albumen, or which contains a large glycocoll-fraction, such as gelatin. A large proportion of the putrefaction in the lower intestine must be attributed to the protein contained in the mucous secretions of the intestine itself. Thus Whipple has shown that toxic proteoses of bacterial origin may be absorbed from an isolated loop of intestine, from which the contents have previously been removed. Hence reduction of the protein intake only reduces one, and not necessarily the larger source of intestinal putrefaction, and the reduction of ethereal sulphates to one-half, by a reduction of protein intake to one-fifth, is probably the utmost that could be expected. We may, therefore, ascribe to the ethereal sulphates, as to the inorganic sulphates, a primarily exogenous origin.

Another channel of sulphur excretion is the **Bile**, wherein sulphur

is contained in the form of **Taurine**, which, combined with cholic acid, forms the taurocholic acid fraction of the mixed bile-acids. Taurine is amino-ethyl sulphuric acid, and its relationship to the sulphur-containing amino-acid of the tissue-proteins, **Cystine**, is shown in the following formulæ:



Cystine.



Taurine.

The taurine thus excreted is mainly reabsorbed and either reëxcreted as taurocholic acid or else transformed into products which are eliminated in the urine. It will be observed that the relationship of taurine to cystine is a very simple one, decarboxylation and oxidation of the sulphur serving to convert the cystine into taurine. This being the case it is of very great interest to note that the excretory products to which these compounds give rise are very diverse, for as Salkowski originally showed, and his results have been confirmed and amplified by Schmidt, von Adelung and Watson, the administration of taurine in large doses to man by mouth, or subcutaneous or intravenous injection, leads to a large increase in the **Neutral Sulphur** output, over eighty per cent. of the taurine being excreted within twenty-four hours in a "neutral" form which Salkowski has identified as **Taurocarbamic Acid**. Now the administration of cystine in moderate dosage, or of polypeptides containing cystine, leads to an increase in the inorganic sulphates only, and a very large dosage is required to elicit an increase of neutral sulphur.

The fact that the administration of cystine, whether by mouth or intravenously, results in an increased output of inorganic sulphates suggests that a portion of the endogenous sulphur metabolism may be represented in the inorganic sulphates, for, as we have previously argued in connection with a possible endogenous origin of urea, if the circulating amino-acids stand in equilibrium with the tissue-amino-acids, as the results of Van Slyke indicate, and these latter in equilibrium with the tissue-proteins, then the disintegration-products of tissue-proteins must be the amino-acids themselves, for otherwise protein synthesis would go on indefinitely and unchecked. But the amino-acids, including cystine of course, when once released from the tissues must be thrown into the common supply and undergo their share of exogenous metabolism. Indeed it may be questioned whether the neutral sulphur output really represents the metabolism of cystine in the tissues of the body considered collectively, or whether it does not possibly represent the destruction of a special fraction of the cystine which is converted by the liver into taurine, and a series of products obtained from the sulphur-containing compounds of the nervous system, cartilage, etc., in which sulphur is present in radicals other

than cystine. Among the constituents of the neutral sulphur fraction may be enumerated **Sulphocyanides** which are found in traces in the urine and also in the **Saliva, Chondroitin-sulphuric Acid**, and a number of poorly-defined nitrogenous acids which have been designated the **Oxyproteic Acids.**

In rare instances **Cystine** is found to occur in the urine in notable quantities, as much as 0.5 to 1.5 grams being excreted in one day. This condition, known as **Cystinuria**, is a much more serious abnormality than alcaptonuria, which it resembles in being due to a defect of metabolism, because the large excretion of this sparingly soluble amino-acid often leads to the formation of deposits or calculi in the bladder. According to Garrod, cystinuria is a rarer disease than alcaptonuria, but it reaches the physician more frequently because of the serious nature of the symptoms which arise. The failure to oxidize cystine, which is characteristic of the cystinuric patient, frequently extends to other amino-acids, and amines, such as **Cadaverine** and **Putrescine**, derived from the decarboxylation of **Lysine** and **Ornithine** may also appear in the urine, and occasionally, leucine and tyrosine. In such cases cystinuria is evidently an expression of a general defect of the deaminizing-mechanism.

An experimental cystinuria may be induced in animals by the administration of halogen-benzenes, such as monochlorbenzene or monobrombenzene. The halogen-benzene is paired with cystine and excreted in this form as **Mercapturic Acid**, in combination with glucuronic acid. The excretion of cystine in these cases is accompanied by a diminution of the output of inorganic sulphates.

The presence of cystine in the urine may be suspected if hexagonal crystals are deposited which are soluble in ammonia and insoluble in acetic acid. If a few crystals are dried, placed on a slide and covered with a cover-glass underneath which is introduced a drop of strong hydrochloric acid, as each crystal is touched by the acid a cluster of fine prisms is seen to spring from it, consisting of cystine hydrochloride (**Wollaston's Test**).

In passing it may be stated that the **Phosphorus** of the diet is wholly or almost wholly excreted in the form of phosphates in the urine and the feces.

URINARY PIGMENTS.

A variety of urinary pigments have been described by different investigators, but only three pigments have been definitely characterised. These are **Urochrome**, a pigment to which the yellow color of urine is mainly due, **Urobilin** which is voided in the form of a colorless chromogen, **Urobilinogen**, which is converted into urobilin by exposure to air under the influence of light, and **Uroerythrin**, which is frequently but not invariably present.

On saturating urine with ammonium sulphate, urochrome remains in solution while urobilin is precipitated. When a solution of urobilin

is dissolved in ammonia and a little zinc chloride solution is added the mixture turns red with a green fluorescence; urochrome, on the contrary, does not yield fluorescent solutions.

Both of these pigments are closely related to the bile-pigments and, therefore, to hemoglobin. They yield the pyrrole reactions and strongly resemble substances which are obtainable from **Bilirubin** by reduction. Urobilin, or its parent-substance urobilinogen is a constant constituent of the feces, but before the identity of the two pigments was realized the urobilin in the feces received a separate name, **Stercobilin**. The quantity of these pigments in the urine is distinctly increased in all fevers, also in hemorrhage and in conditions involving the destruction of red blood-corpuscles, and in diseases of the liver.

Uroerythrin is the pigment which frequently gives a red color to urinary sediments, particularly to sediments of uric acid, which, owing to its presence, may appear like grains of cayenne pepper. It does not yield fluorescent solutions and is rapidly decolorized by light. The normal color of solutions is pink, but strong sulphuric acid changes this to carmine, and alkalies to green. Uroerythrin is believed not to be related to bilirubin but to be derived from **Skatole**. The quantity is increased by muscular activity, profuse perspiration, alcohol, immoderate eating, fevers and diseases of the liver.

The presence of urobilinogen in the feces and the probable derivation of uroerythrin from skatole render an alimentary origin of these pigments very probable. It is likely that urochome and urobilin arise by bacterial decomposition of the bile-pigments in the lower intestine. In confirmation of this view it is found that strong **Intestinal Putrefaction** leads to an increase of the urobilin output while exclusion of bile from the intestine reduces the output to zero. If the exclusion of bile from the intestine be due to mechanical occlusion of the bile-ducts, then bile-pigments, but not urobilin, appear in the circulation and in the urine.

THE PROPERTIES AND COMPOSITION OF URINE.

The volume of the urine which is voided daily necessarily varies very greatly with the quantity of water which is drunk, the quantity of water contained in the food, the amount of fluid lost from the body by perspiration and a variety of other factors such as the presence or absence of **Diuretics** such as **Caffein** or **Theobromin** in the diet, or hyperactivity of the posterior lobe of the pituitary body which may lead to a chronic hypersecretion of a dilute urine containing no sugar; a condition known as **Diabetes Insipidus**.

The **Specific Gravity** of the urine necessarily varies with its volume, usually fluctuating between 1.008 and 1.030. The reaction is usually acid, but immediately after a meal an alkaline reaction, the "alkaline tide" may frequently be observed, and on a purely vegetable diet the urine is not infrequently alkaline. The sulphur and phosphorus

in the proteins of a meat-diet are oxidized wholly or in part to the highly dissociated sulphuric and phosphoric acids which decrease the alkali-reserve of the blood and tissues and are excreted as acid salts in the urine, while the alkaline salts in vegetables are oxidized to carbonates or bicarbonates and excreted as such.

According to Fitz and Van Slyke the titratable acidity of the urine (employing phenolphthalein as an indicator) runs remarkably parallel, in conditions of **Acidosis**, with the decrease of the alkali-reserve. In order to observe this parallelism, however, we must add to the titratable acidity the amount of **Ammonia** in the urine which has been furnished by the tissues as a means of neutralizing a portion of the excess of acid. This can be estimated by the method of Sörensen, the **Formol Titration**, which depends upon the fact that formaldehyde in faintly alkaline solutions unites with ammonia to form hexamthylene-tetramine, which has a neutral reaction:

$$4\,NH_4Cl + 6\,HCHO + 4\,NaOH = N_4(CH_2)_6 + 10\,H_2O + 4\,NaCl$$

The urine is first rendered very faintly alkaline to phenolphthalein, then neutral formaldehyde is added and the quantity of alkali which must be added to render the urine alkaline again is determined by titration. This is equivalent to the ammonia which has been converted into hexamethylene-tetramine.[1]

The relationship observed by Fitz and Van Slyke is expressed by them in the following formula, which is an adaptation of the formula of Ambard for the excretion of urea and chlorides:

$$\text{Bicarbonates in the plasma} = 80 - \sqrt{\frac{D}{W}\sqrt{C}}$$

where D is the titratable acidity plus the ammonia output, W the weight of the individual and C the concentration of acids in the urine, or $\frac{D}{V}$, where V is the volume of urine. The figure 80 represents the maximum yield of carbon dioxide in volumes per cent. which may be obtained by treating blood-serum with sulphuric acid. Reduction of the alkali-reserve below this point results in the urinary excretion of an excess of acid radicals which is expressed by the factor:

$$\sqrt{\frac{D}{W}\sqrt{C}}$$

This relationship is purely empirical and the agreement between the calculated and observed values of the alkali-reserve cannot be relied

[1] The $-NH_2$ groups of amino-acids will react with formaldehyde in the same way as ammonia. The concentration of amino-acids in the urine is so small, however, that, as a rule it may be neglected.

upon to within ten per cent. It nevertheless is of value as serving to show that titratable acidity of the urine, if added to the ammonia, or *protective* basic output, is a real indication of the presence or absence of acidosis.

We have seen that the diurnal output of most of the nitrogenous excreta is profoundly influenced by the diet. No normal composition of the urine can therefore be formulated which is not subject to wide fluctuations which are nevertheless within the limits of diversity which may be exhibited by a single normal individual under varying dietary conditions. The following may, however, serve to illustrate the composition to which the urine of a normal individual subsisting upon a moderate and mixed diet would more or less closely approximate:

NORMAL COMPOSITION OF URINE.

(Illustrative Analysis.)

The following represents a normal twenty-four-hour sample of urine of volume 1500 c.c. and specific gravity 1.010–1.015:

Constituent.	Weight in grams.	Approximate percentage.
Water	1440.0	96.0
Solids	60.0	4.0
Urea	35.0	2.33
Uric acid	0.75	0.05
Hippuric acid	0.7	0.05
Oxalic acid	0.015	0.001
Aromatic oxy-acids	0.06	0.004
Creatinine	1.0	0.07
Thiocyanic acid (as KSCN)	0.15	0.01
Indican	0.01	0.001
Ammonia	0.65	0.04
Sodium chloride	16.5	1.10
Phosphoric acid (P_2O_5)	2.5	0.15
Total sulphuric acid	2.5	0.15
Silicic acid	0.45	0.03
Potassium (K_2O)	2.5	0.15
Sodium (Na_2O)	5.0	0.30
Calcium (CaO)	0.25	0.015
Magnesium (MgO)	0.30	0.02
Iron	0.005	0.0004

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PART VI.

THE ENERGY-BALANCE OF THE ORGANISM.

CHAPTER XXIII.

THE ANIMAL BODY AS A MACHINE.

THE APPLICABILITY OF THE LAW OF THE CONSERVATION OF ENERGY TO LIVING ORGANISMS.

To all of our not very remote forebears and to the majority of those of our contemporaries who vote, legislate and govern in this our present day, Life was, or is, a thing apart from the Universe, independent of cosmic laws, controlling rather than expressing the forces of nature. The inversion of this primitive idea which was ultimately to result in the attainment of our present conception of life, as the outcome of forces which it does not of itself create, originated in the investigations of that greatest of French chemists, Lavoisier.

The clue to the true nature of the processes of *combustion* had previously been provided by the discovery by Priestley that air contains a substance which is essential to combustion and is consumed thereby. It was Lavoisier, however, who showed that this gas is absorbed by and becomes combined with the burning substance, and the amplification of this discovery led to the enunciation of the law of the **Conservation of Matter.** The corresponding law in the domain of energy-transformation was not formulated until 1845, over fifty years later. Nevertheless it is to Lavoisier also that we must accredit the investigations which first established the applicability of the law of the **Conservation of Energy** to animals. It has frequently happened in the history of scientific investigation, that a truth which was not generally apprehended or clearly enunciated at the time has nevertheless been tacitly assumed in advance of their period by investigators possessing exceptional powers of insight and discovery. It is a mistake to suppose that successful scientific discovery is the outcome of purely logical processes of thought in the mind of the investigator. The great discoverer appears to be distinguished from equally diligent but less successful investigators quite as much in his possession of a

species of intuitive sympathy with the order of nature, as in his purely intellectual endowments as these are ordinarily understood. There can be no question at all that both Lavoisier and Faraday, without ever having formulated it in so many words, and certainly without adequate proof of its validity, nevertheless assumed the truth of the law of the conservation of energy and were guided in their investigations by this assumption.

Lavoisier had shown in 1790 that the oxygen absorbed and transformed into other substances by a man or animal is increased by the performance of **Muscular Work** and by exposure to a low temperature. Work and the production of **Bodily Heat** were thus correlated with the occurrence of chemical reactions which were known to liberate energy, *i. e.*, combustions. The next step was to institute a direct comparison between the heat of combustion of a carbonaceous material and the heat-evolution of an animal, a comparison which has since then been repeated many times, and with ever-increasing exactitude. The material chosen by Lavoisier as a standard for comparison was pure carbon. He measured the amount of heat evolved in the conversion of the carbon into carbon dioxide, and he then measured the amount of heat and carbon dioxide given off by a guinea-pig in a period of ten hours. The heat-evolution was estimated from the latent heat of ice which was melted by the heat of the burning carbon in the one experiment and by the heat of the animal's body in the other. It was found that the guinea-pig communicated 31.8 calories to the ice, while 25.4 calories were yielded by burning enough carbon to furnish the amount of carbon dioxide exhaled by the animal in the same period. The figures are not equal and we now know why. Apart from experimental errors arising from the unavoidably imperfect technic of the estimation, the animal burnt, not only carbon during the period of its incarceration in the ice-chamber, but also hydrogen. Were **Carbohydrates**, in which the hydrogen is fully neutralized by oxygen already present in the molecule, the sole source of energy, then the comparison instituted by Lavoisier would have been adequate, but the **Fats** and **Proteins** contain an excess of hydrogen, of which the heat of combustion must be added to that of the carbon in order to establish the chemical origin of animal heat and work. Nevertheless the figures obtained by Lavoisier were sufficiently comparable to afford decided encouragement to the view which he himself expressed: "La vie est une fonction chimique."

In 1793 Lavoisier was condemned to death and executed by the apostles of Liberty, Equality and Fraternity. His crime appears to have consisted in his being a man of superior intellect and education who had dared to express his opinion that the French Academy of Sciences should be preserved and not suppressed, as the National Convention desired. His appeal for liberty to live and serve was thus answered by the president of the tribunal which condemned him:

"La République n'a pas besoin de savants"—which was true, until 1870, let us say, or 1914. It is to a Roman politician that we owe the very popular and oft-quoted doctrine that "The Voice of the people is the Voice of God." On this occasion the spokesman of the people assured one of the greatest discoverers that humanity has produced, that a republic had no need of him or of his kind. To a Swedish physicist, Oersted, we owe a different doctrine, which he expressed in these words: "The Laws of Nature are the Thoughts of God." If we should estimate the value of these two doctrines by their fruits, then doubtless we would prefer the doctrine of the physicist who produced telegraphy to that of the demagogue who planned a brutal and senseless murder. Contemporary events will doubtless, in time to come, furnish us with an abundance of additional means of estimating the relative value of these theories.

The work which had been thus initiated by Lavoisier, was carried on by his pupil Liebig, who, however, mainly devoted his attention and his life's work to the firm establishment of the Law of the **Conservation of Matter** in its application to living organisms. The methods of organic analysis which he devised, and the investigations which he undertook, laid the foundations of analytical biochemistry as we know it today. The energy-transformations of life were destined to become the preoccupation of Liebig's pupil, Voit, and of a series of investigators who owed to Voit their inspiration. Thus, to the second and third generations of investigators succeeding Lavoisier, fell the task of achieving the fruition of his labors.

In order to render possible an accurate comparison of the kind which was attempted by Lavoisier it was first of all necessary to ascertain **Heats of Combustion** of the various foodstuffs. The actual fuels burnt by the animal machine are carbohydrates, fats and proteins, and it is evidently with the heat of combustion of these substances, and not merely that of carbon, that we should compare the heat-evolution of an animal.

The **Calorific Values** in heat-units per gram for the different representatives of the three main classes of foodstuffs do not vary greatly among themselves. The molecules of the **Fats** and **Proteins** are so large that the differences of composition or structure which they display affect the total heat of combustion but slightly, while the **Carbohydrates** uniformly contain the proportion of oxygen which is requisite to burn their hydrogen and hence the combustion-value for each carbohydrate is very nearly proportional to the carbon which it contains and this in turn is proportional to the weight of the molecule. The following are the calorific values of various foodstuffs, as estimated by complete combustion in a calorimeter, the heat-output being expressed in terms of the large calorie, or quantity of heat required to raise the temperature of one kilogram of water from 0° C. to 1° C.

	Cals.
Proteins:	
Casein	5.86
Egg-albumin	5.74
Serum-albumin	5.92
Average	5.84
Fats:	
Tissue-fat	9.48
Butter-fat	9.23
Olive oil	9.33
Average	9.35
Carbohydrates:	
Glucose	3.74
Cane-sugar	3.96
Milk-sugar	3.95
Maltose	3.95
Starch	4.18
Average	3.96

The figures usually employed for the fats and carbohydrates as they actually occur in a mixed diet are those which were originally estimated by Rubner, namely:

One gram of fat = 9.3 calories
One gram of carbohydrate = 4.1 calories

the high value for carbohydrates being employed on account of the predominance of starch among the carbohydrates of an ordinary mixed diet.

The heat-value of carbohydrates and fats for the body must be the same as that indicated by the combustion-calorimeter, since the products of combustion are in both cases identical, namely, carbon dioxide and water. The case is far different for the **Proteins**, however, because these are not completely burnt, the nitrogen being excreted in the form of urea, creatinine and so forth, which are substances still capable of yielding heat when they are completely oxidized. Furthermore, the proteins as they actually occur in the diet are not completely digested and assimilated, a proportion of indigestible or difficultly assimilable material being evacuated in the feces. The true heat-value of protein to the animal body is therefore not indicated by the combustion-calorimeter.

The determination of the actual calorific value of protein in the animal body was first carried out by Rubner. His procedure was as follows: The calorific value of dried muscle-tissue was determined in the combustion-calorimeter, and the heat-values of the urine and feces upon an exclusive meat-diet were also determined. Subtracting the heat-value of the excreta from that of the food, and also a small correction representing the heat of solution of the urea in the urine, it was found that an average of about 4.1 calories per gram was actually available to the animal from the protein in its diet. The

actual calorific value of a protein to an animal is therefore the same as that of a carbohydrate, both being far inferior in heat-value to the fats.

The necessary data for the accurate evaluation of the comparison which Lavoisier attempted were by now assembled and the comparison, when actually carried out by Rubner in 1894, established beyond any doubt the validity of the principle of the **Conservation of Energy** in the phenomena of life. The experiments were carried out upon a dog, because there existed at that time no calorimeter, of sufficient size to contain a man, which would accurately measure the heat evolved during a period of twenty-four hours. The heat actually imparted by the dog to the calorimeter in twenty-four-hour periods was measured and this was compared with the heat-value of its food computed from the nitrogen in the urine (1 gram Nitrogen = 6.25 grams protein = 25.63 calories) and from the output of water and carbon dioxide. The following are the details of his comparisons, the "food" in starvation consisting, of course, of the proteins and fats of the animal's own tissues:

Food.	Number of days.	Heat calculated from metabolism.	Heat directly determined.	Difference in percentage.
Starvation	5	1296.3	1305.2	−1.42
	2	1091.2	1056.6	
Fat	5	1510.1	1498.3	−0.97
Meat and fat	8	2492.4	2488.0	
	12	3985.4	3958.4	
Meat	6	2249.8	2276.9	−0.42
	7	4780.8	4769.3	+0.43

When one considers the complexity of these estimations, the multitude of factors which participate in determining their outcome, and the elaborate character of the apparatus employed, the coincidence of the calculated and actual output is so exact as to leave no room for doubt that the law of the conservation of energy applies no less to animals than to other machines. The energy which the animal dissipates is derived from the combustion of foodstuffs, just as the energy dissipated by a locomotive is derived from the oxidation of its fuel. In the living, as in the inanimate machine, the potential energy of the fuel is released by oxidation and reappears in the form of heat and work.

An even more exact balance between income and output was however sought for and found by the American investigator, Atwater. The extraordinary degree of accuracy which was attained in his investigations was rendered possible by the invention of the **Atwater-Rosa Calorimeter**, which was of sufficient capacity to hold a man and yet so technically perfect that when a measured amount of heat was generated within the calorimeter by an electric current, the quantity of heat liberated could be measured to within 0.01 per cent. (Figs. 48 and 49). The amount of protein burnt by the subject was estimated

from the nitrogen in the urine and in the feces. The carbon which would be derived from this quantity of protein was deducted from the total carbon output and the difference yielded the total non-protein carbon, or carbon derived from carbohydrates and fat. The carbohydrates in the food were measured and the corresponding quantity of carbon deducted from the total non-protein carbon. The difference represented carbon derived from the fat. In this way the quantities of each of the three classes of foodstuffs consumed were estimated and

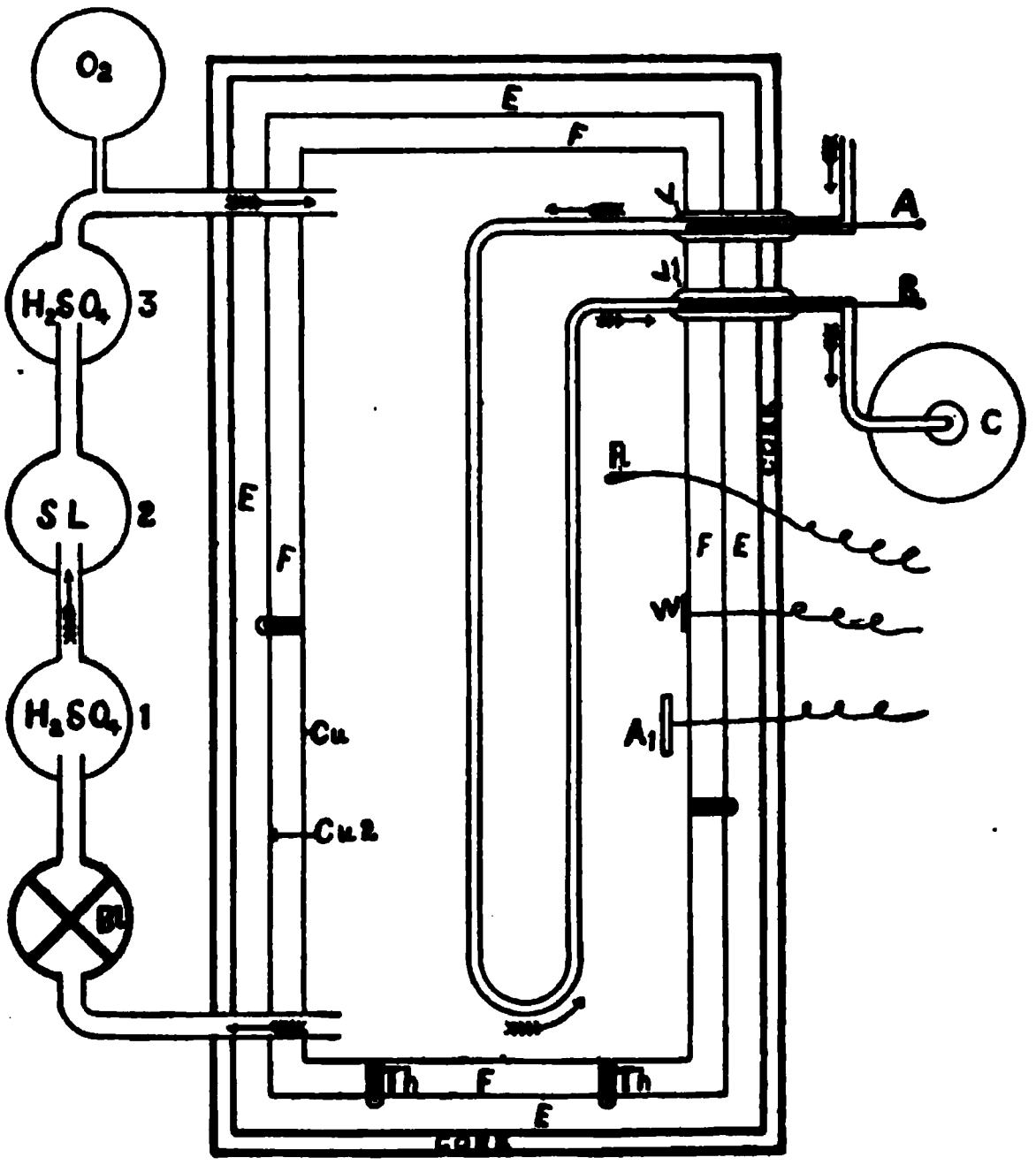

FIG. 48.—Schematic diagram of the Atwater-Rosa-Benedict respiration-calorimeter. O_2, oxygen introduced as consumed by subject; *3*, H_2SO_4 to catch moisture given off by soda-lime; *2*, soda-lime to remove CO_2; *1*, H_2SO_4 to remove moisture given off by subject; *Bl*, blower to keep air in circulation; *V*, vacuum jacket; *C*, tank for weighing water which has passed through calorimeter each hour; *W*, thermometer for measuring temperature of wall; A_1, thermometer for measuring temperature of the air; *R*, rectal thermometer for measuring temperature of subject. (After Lusk.)

the energy which their combustion could yield was computed in the manner indicated above and compared with the actual heat-evolution of the subject. The results of forty days' experimentation with three different subjects yielded the following averages:

Calculated daily output	2717 calories
Observed daily output	2723 "

Difference, 0.2 per cent.

A further refinement of technic consisted in the simultaneous estimation of the carbon-dioxide output and the oxygen intake, from

which the **Respiratory Quotient** could be calculated. Deducting the protein carbon from the total carbon output, and the oxygen required to oxidize the protein from the total oxygen intake, the ratio of the non-protein carbon dioxide to the residual oxygen intake, or the non-protein respiratory quotient; afforded a measure of the proportion of fat to carbohydrate actually consumed by the subject of the experiment. Thus a non-protein respiratory quotient of 0.707 indicates the

FIG. 49.—General view of the respiration-calorimeter laboratory at Middletown, Connecticut. The calorimeter-chamber is seen, with window open upon the right. The principle of its construction is that of an ordinary refrigerator, namely, a chamber surrounded by a series of confined air-spaces. The inner chamber is of copper. This is succeeded by a wall of zinc and two walls of wood, each pair of walls being separated by about three inches of air-space. Gain or loss of heat through the metallic walls of the chamber is prevented by keeping the zinc wall at the same temperature as the copper. Any difference of temperature between these two walls is indicated by a thermocouple and a galvanometer. Heat is supplied to the air-space surrounding the zinc wall by passing an electrical current through coils of resistance-wire. Cooling is accomplished by currents of water. The heat generated in the chamber is removed partly in the form of the latent heat of vaporization of the water exhaled from the lungs and partly by means of cold-water absorbers. The quantity of heat evolved is computed from the amount of water passing through the heat-absorbers and its rise in temperature during its passage. (After Benedict and Milner.)

combustion of pure fat, a quotient of 1.00 indicates the combustion of pure carbohydrate (cf. Chapter XXII) and intermediate values represent the combustion of a mixture of these foodstuffs, the composition of which can be estimated by a simple calculation.

It now remained, in order to complete the demonstration of the validity of the law of the conservation of energy in the animate world, to investigate the source of the energy which is expended by an animal in the performance of external work. In the experiments hitherto

enumerated the subjects were at rest, and although their respiratory and cardiac muscles were contracting and the skeletal muscles maintained in tone or even contracting, yet, the whole of the organism being enclosed within a heat-insulated system, the effect of all these movements ultimately appeared and was estimated in the form of heat. The case is different when, as in many of Atwater's experiments, the subject was made to perform external work, by operating a stationary bicycle which was so arranged that the rotation of the wheels raised a weight. The energy output was not in this case expressed entirely in the form of heat, but in part in the form of **Mechanical Work.** We can express this work in terms of heat-units, however, just as we can express heat in terms of electrical units or electrical units in terms of mechanical work again. Since no energy is ever lost and all forms of energy are equivalent to one another, the heat-value consumed in performing mechanical work can be directly calculated from the known mechanical equivalent of heat. The following are the results which Atwater obtained in the investigation of this problem:

		Calories.		
	Days.	Income per twenty-four hours.	Output per twenty-four hours.	Difference. per cent.
Rest experiments:				
7 experiments with E.O. .	25	2268	2259	−0.4
1 experiment with A.W.S.	3	2304	2279	−1.1
3 experiments with J.F.S.	9	2118	2136	+0.8
1 experiment with J.C.W.	4	2357	2397	+1.7
Average . . .	41	2246	2246	±0.0
Work experiments:				
2 experiments with E.O. .	8	3865	3829	−0.9
4 experiments with J.F.S.	12	3539	3540	±0.0
14 experiments with J.C.W.	46	5120	5120	±0.0
Average . . .	66	4682	4676	−0.1

To within one part in a thousand the output of heat plus work was equal to the calorific value of the foodstuffs consumed. We can hardly doubt that this minute discrepancy was of purely technical origin and that these experiments represent the culmination of the proof which Lavoisier had sought a hundred years previously, that the energies of life are derived simply and solely from the chemical energy of the foodstuffs.

The fundamental importance of these investigations cannot be overrated, for they reveal to us in the clearest possible manner the fact that life is the outcome of a complex of forces which it does not create. We are enabled by them to confidently state that if there is such an entity as "**Vital Force**" created and generated out of nothing by living organisms, then the inconspicuousness of its effects is commensurate with the inconspicuousness of its origin. They must be confined to somewhat less than a one thousandth part of the total activity of the organism.

We must be careful, however, in formulating any such fundamental conclusion not to go too far. We must beware of overstepping to the slightest extent the sure ground of fact which our evidence affords, and we must therefore candidly admit that while the evidence accumulated by the remarkable series of investigations which we have briefly and inadequately outlined, clearly justifies the conclusion that no source of energy is contributed to or resides in the organism that is not comprised in the chemical energy of its foodstuffs, and the heat of its environment, yet we cannot definitely reject the possibility that forces of evanescent magnitude which are not comprised in either of the above categories may influence, in the manner of a catalyzer, the *rate* of discharge of energy from the organism. We cannot disprove this, but then, on the other hand, if one should choose to assume the existence of such forces the burden of proof clearly rests upon the originator of the hypothesis. In the interpretation of life-phenomena, so far as we have as yet been enabled to subject them to measurement, such an assumption has proved to be altogether unnecessary, and hence our present state of knowledge affords for it no foundation whatever. No sure ground is possible in scientific discovery unless we proceed from the known to the unknown. The assumption that hitherto unknown forces are involved in life cannot assist but only retard its interpretation until and unless every previously known possibility has been exhausted in a vain endeavor to reconcile the facts. But the existence of unknown possibilities manifestly cannot be contradicted upon *a priori* grounds, and a dogmatic insistence upon the sufficiency of the known has only too frequently, in the history of science, served but to pave the way for a subsequent recantation.

THE ISODYNAMIC VALUES OF THE FOODSTUFFS.

Since the products of the combustion of the **Fats** and **Carbohydrates** in the diet are the same, namely carbon dioxide and water, it was suggested at an early period in the investigation of metabolism that these components of the dietary might be mutually interchangeable in equicalorific quantities. This possibility was experimentally realized by Rubner, who found that 100 grams of fat in the diet could be replaced by 232 grams of starch or 234 grams of cane-sugar, the equicalorific values estimated from the heat of combustion being 229 grams of starch and 235 grams of cane-sugar. The same conclusion was ultimately reached by Atwater in a series of experiments in which the subjects were made to perform external work, so that part of the energy of the foodstuffs had to be expended for this purpose. The procedure of the experiments was designed to test the efficacy of the fats as substitutes for carbohydrates in a variety of ways. Thus the diet was insufficient to maintain bodily equilibrium, so that there was a loss of weight throughout the duration of the experiments due to the consumption of the subject's tissues. The loss of body-substance on the diet

containing carbohydrates could thus be compared with that experienced on the diet containing fats, and the relative value of these constituents of the dietary as tissue-sparers could thus be estimated. The external **Mechanical Work** performed in both sets of experiments was as nearly as possible the same, and equivalence of total energy-consumption on the two diets would therefore indicate equal availability of fats and of carbohydrates for the performance of mechanical work. The following table summarizes the results of these experiments:

Experiment number.	Time, days.	Heat derivable from food, calories.	Heat equivalent of external work, calories.	Total energy-output, calories.	Calories equivalent to gain (+) or loss (−) of tissue.
40 J.C.W. carbohydrate-diet .	4	4180	518	5251	−1071
41 J.C.W. fat-diet	4	4150	522	5304	−1154
44 J.C.W. carbohydrate-diet .	4	4602	571	5125	−523
43 J.C.W. fat-diet	4	4496	548	5155	−659
47 J.C.W. carbohydrate-diet .	4	4366	562	5173	−807
46 J.C.W. fat-diet	4	4478	551	5193	−715
53 J.C.W. carbohydrate-diet .	3	5132	587	5104	+28
52 J.C.W. fat-diet	3	5120	607	5309	−189
Average of four experiments with carbohydrate-diet	15	4532	558	5167	−635
Average of four experiments with fat-diet	15	4524	554	5236	−712

The substantial equivalence of the fats and carbohydrates as sources of heat and work and sparers of tissue in these experiments is evident. There is some indication that the loss of tissue on the fat-diet is greater than it is on a carbohydrate-diet, and this is especially evident in the experiment in which the total calorific value of the diet was relatively high. The reason for this probably lies in the fact that, as Zeller has recently shown, if the preponderance of fat over carbohydrates in the diet be too great, even when the total calorific value of the diet is kept constant, acetone bodies appear in the urine and an **Acidosis** arises necessitating the production of **Ammonia** by the tissues tc neutralize the excess of acid radicals in the blood. The output of nitrogen is consequently increased and loss of body-substance accelerated. This effect only appears in normal individuals, however, when less than ten per cent. of the total calories are given in the form of carbohydrate. Up to this limit, therefore, the carbohydrates in the diet may be replaced by fat without influencing very appreciably the total heat-output or wastage of tissue-materials. In **Diabetes**, of course, the limit of tolerance for fats is much lower than this.

In the replacement of the fats by carbohydrates we are limited in another direction. So far as the mere question of heat-equivalence is concerned the complete replacement of the fats in the dietary by carbohydrates is doubtless entirely feasible, more especially since the conversion of carbohydrates into body-fat is a regular concomitant of

insufficient utilization of the carbohydrates of the diet for the production of heat and work. We have seen (Chapter XX), however, that certain essential **Substrates of Growth**, or raw materials for the synthesis of protoplasm are contained in the animal fats and, so far as we are yet aware, in no other abundant constituents of the diet. The total replacement of fats by carbohydrates, therefore, is likely to result in unbalanced tissue-waste through the lack of non-synthesizable atom-complexes which do not necessarily contribute any appreciable share to the energy-output. The total replacement of animal fats by **Vegetable Oils** is for a like reason impracticable. The proportion of animal fat which is requisite for maintenance is, however, very small, and provided this small residuum is retained, the fats of the dietary may be replaced by carbohydrates in equicalorific proportions without affecting the balance of energy-input and -output.

In the case of the **Proteins** a number of complications arise which limit in a variety of directions the application of the principle of isodynamic values. In the first place the proteins are the medium through which the body acquires its nitrogen. Their complete replacement by fats or carbohydrates is therefore obviously impossible. Then, again, different types of protein are not even isodynamic with each other, for those which lack or are deficient in certain amino-acids, such as **Gelatin, Zein** or **Gliadin** will not replace the protein in a normal mixed diet however great an excess of the incomplete protein may be employed (Chapter XX). No nitrogen balance is possible unless the missing amino-acids are supplied, and upon a diet containing an abundance of nitrogen the output will continuously exceed the intake. If, however, the missing amino-acids are added to these proteins, as, for example, tyrosine, cystine and tryptophane to gelatin, then the attainment of nitrogenous equilibrium becomes possible because all of the constituent parts of tissue-protein are then present in the diet.

Although gelatin cannot replace other proteins in the diet, yet it is possible to attain nitrogenous equilibrium on a smaller amount of normal dietary protein if gelatin be also present. If the total heat-requirement of the normally fed animal be supplied solely in the form of carbohydrates and fats a certain daily loss of nitrogen will occur which is due to the consumption of tissue-proteins. If 7.5 per cent. of the heat-value be now supplied in the form of gelatin the excess of loss over intake is diminished by 23 per cent. If, however, 60 per cent. of the heat-value of the food is supplied by gelatin the saving of tissue-protein is only 35 per cent., and if the whole of the heat-value be supplied in gelatin only 37.5 per cent. of the tissue-wastage is spared. The principle of isodynamic values is therefore manifestly inapplicable to the quantitative relationship between gelatin and the other dietary constituents unless a sufficiency of other protein be at the same time supplied to furnish the full requirement of tyrosine, cystine, and tryptophane.

A further limitation upon the application of the principle of isody-

namic values to the protein constituents of the dietary, arises from the fact that an increase of protein in diet actually stimulates the total metabolism, so that more food is burnt and more heat evolved on a diet high in protein than upon a diet which contains less protein. This phenomenon, which Rubner terms the **Specific Dynamic Action** of proteins, is very well displayed by the effect of administering protein to a starving animal. One might suppose that if a starving animal is losing a certain amount of tissue-protein daily, the administration of this amount of protein daily would suffice to balance the nitrogenous input and output. This is not the case, however, for on increasing the nitrogenous input an increase of nitrogenous output also occurs and the balance remains negative. A further increase of nitrogenous input calls forth a still greater metabolism of protein until, on an exclusively protein diet, a balance between intake and output is attained with an output of nitrogen no less than three and one-half times that which is observed in the starving animal. In man the quantity of protein thus required to obtain nitrogenous equilibrium is greater than he can conveniently consume, and even when nitrogenous equilibrium has been attained the carbon balance remains negative, since not only the nitrogenous metabolism, but the metabolism of fats and carbohydrates is stimulated by protein. The effect of protein is therefore to greatly increase the heat-evolution of the body, and the replacement of fat or carbohydrate by protein in a diet which is just sufficient to maintain equilibrium results in rendering the diet inadequate to replenish the tissue-loss. The proteins cannot, therefore, replace fats or carbohydrates in isodynamic proportions.

The origin of the specific dynamic action of the proteins has been sought by Lusk, who investigated the effects of individual **Amino-acids** upon the heat-output in starving dogs. He found that while glycocoll and alanine greatly increase the production of heat, and leucine and tyrosine slightly, glutamic acid is devoid of action. A mixture of 5.5 grams each of glycocoll, alanine, glutamic acid and tyrosine produced as much increase of heat-output as 100 grams of meat.

THE PROTEIN REQUIREMENT IN THE DIETARY.

From the preceding considerations it must be evident that the proteins are the most wasteful constituents of the dietary, since they increase the consumption of other constituents as well as that of protein itself. The proteins are also the most expensive foodstuffs from a commercial point of view and this is particularly true of the proteins of animal origin, for while there is little wastage of energy or materials in the growth of the vegetable constituents of the diet, a very large wastage occurs in the synthesis of animal proteins for human consumption. An ox or sheep may, for our immediate purpose, be regarded as an ambulatory factory of protein. In order to supply this factory with raw materials, vegetable proteins, carbohydrates and fats must first

be grown at the expense of the constituents of the soil and the preoccupation of space that might be otherwise utilized. Not only must an amount of vegetable food be provided equivalent in heat-value to the animal foodstuffs which we desire to synthesize, but an enormous excess, to supply the radiation of heat and mechanical work performed by the animal throughout the period of its growth. The **Animal Proteins** therefore represent a consumption and expenditure of food-materials totally disproportionate to their calorific value. The vegetable proteins, on the other hand, are also expensive because the proportion of protein in the majority of vegetable tissues, with few exceptions, is extremely small.

As a measure of national economy, therefore, if we view the matter solely from a financial standpoint, a restriction of the protein-consumption to the minimum consistent with health and efficiency would seem to be highly desirable. Now the consumption of protein foodstuffs and particularly of animal proteins varies very greatly among different peoples. The following pre-war statistics are furnished by Ostertag:

	Meat consumed per day per capita, in grams.
Australia	306
United States of America	149
Great Britain	130
France	92
Belgium and Holland	86
Austria-Hungary	79
Russia	59
Spain	61
Italy	29
Japan	25

It will be observed that the consumption of meat in the English-speaking countries far exceeds that which prevails elsewhere. Either the English-speaking countries and particularly Australia are wastefully dissipating their food-values, or else a large proportion of the population of Europe is chronically suffering from suboptimal consumption of protein.

The standard requirement of protein, partially derived from meat and in part from vegetables and cereals, was computed by Voit to be 118 grams for the average man not engaged in heavy labor, and 90 grams for a woman. This estimate was based upon a statistical comparison of the actual consumption by presumably normal persons subsisting upon a mixed diet. The necessity for this intake of protein has of recent years, however, been sharply challenged by Chittenden and others of the American school of physiologists and biological chemists. The statistical method of estimating protein-requirements is based upon the assumption of the exercise of free choice by the individual and the underlying supposition is made that prevailing diets represent a species of "survival of the fittest." It is obvious, however,

that if this criterion were to be applied in Japan it would yield far different estimates from those which would result from its application in England. As Taylor has observed, the customary dietary of different races has in no small degree been fashioned by their ethnological development. "In some lands races were compelled to adopt cultivation of the soil, in other places, fishing, in some areas the chase remained, long into relative civilization, one of the chief methods of securing food. The variations in ethnological development brought about by enforced cultivation of the soil, as contrasted with the state of affairs in a tribe of hunters, are well illustrated in different tribes of our American Indians. Depending upon the method of sustaining the life of the tribe, the standard diet of the tribe varied. Only under modern conditions of transportation have the instincts and tastes of man had opportunity for full choice in diet. Compulsion to some extent and in some degree there has always been."

Chittenden was able not only to maintain nitrogenous and calorific equilibrium for prolonged periods on a much lower protein intake than that recommended by Voit, but he was able to keep athletes in a condition fitting them for extreme exertion. According to Taylor the **Nitrogenous Metabolism** of a man of 70 kilos may be summarized as follows, the nitrogenous output being expressed in terms of grams of protein:

	Grams per day.
Nitrogen output on protein-free diet with carbohydrates	10 to 15
Nitrogen output in starvation, lowest level	15 to 20
Nitrogenous and caloric equilibrium, with ample ingestion of carbohydrate	30
Nitrogenous and caloric equilibrium, largely with fat	40
Normal protein input, safety margin of 100 per cent.	70
Nitrogenous and caloric equilibrium on a pure protein diet	750

Nitrogenous and calorific equilibrium can therefore be attained on a diet rich in carbohydrates with a daily intake of only one-third of the amount of protein recommended by Voit. It cannot be positively affirmed that this low protein intake would also suffice to permit normal growth in children or adolescents. It has been argued that as a great part of even this small protein intake is simply deaminized and burnt in the **Exogenous Metabolism** there must be plenty to spare for tissue-synthesis. It has never been demonstrated, however, that the exogenous metabolism is reducible below a certain level. In fact the deaminization of amino-acids with production of urea continues even in starvation. There is apparently, in so far as protein is concerned, no level of the nutrient-reservoir at which a large overflow does not occur. If the overflow and inflow are nearly balanced and the overflow (*i. e.*, exogenous metabolism) is irreducible upon a diet of given composition, then it is clear that the outflow of nutrients to the tissues may be just sufficient to maintain repair and yet quite inadequate to synthesize additional tissue, despite the fact that the intake is far above

that which would, in the absence of the overflow, be necessary for this purpose. In order to establish the adequacy of a maintenance-income of protein for growth it would be necessary to show that the rate of exogenous metabolism, which appears to be governed, at least in part, by the **Thyroid**, is reduced when tissue-accretion occurs. The experimental indications are quite the reverse and tend to show that tissue-accretion is not a cause, but may be a consequence of lowered exogenous metabolism.

It is clear, however, that adults may maintain themselves in nitrogenous and calorific equilibrium upon a much lower protein intake than is customary in many countries, and the question therefore arises whether a restriction of the protein intake, particularly in the English-speaking countries, may not be nationally and economically desirable. We should be cautious in deciding this question upon an insufficiency of evidence. A multitude of factors enter into the question besides the merely financial factor. In the first place it may be stated that no harmful effect of a high protein diet in normal persons has ever been demonstrated. No particular disease is noticeably more common among people accustomed to a high protein intake than among those accustomed to a low protein intake. On the contrary diseases traceable to lowered resistance of the peripheral tissues, such as **Trachoma**, are decidedly more abundant among people whose diet is deficient in protein, although it must be admitted that the dietary of these peoples is probably deficient in other respects beside that of protein-content. A high protein intake does not throw a "load upon the kidneys" which is deleterious in normal persons, and in any case the "load" is very easily lightened by a copious intake of water.

On the other hand, taking Australia as an extreme instance of a community which is accustomed to a high protein intake, we find from the pre-war statistics of the Commonwealth Government that the **Death-rate** was extraordinarily low, nearly one-half that which prevailed in Italy and Austria, lower in fact than in any other country excepting New Zealand, which is also a community of high protein consumption. The **Cancer** death-rate was intermediate between that of Italy and that of France, two communities each consuming far less meat per capita than the Australian.[1] The birth-weight of Australian infants of British parentage exceeds that of British infants born in England by over ten ounces.[2] No trace of deleterious influence of the high proportion of meat in the dietary is thus perceptible. On the other hand the diversity of climatic and social and economic conditions forbids us from drawing the opposite conclusion that the high protein intake is positively beneficial.

It may be pointed out, however, that an unusually low, and also an exceedingly high rate of **Exogenous Metabolism** are alike deleterious to

[1] Official Year-book of the Commonwealth of Australia, 1914.

[2] T. Brailsford Robertson: University of California Publications, Physiology, 1915, 4, p. 207. Amer. Jour. of Physiol., 1915, 37, p. 1.

the general welfare and efficiency. Physicians seek in some instances to correct the former condition by the administration of thyroid extract or of other preparations which are believed to stimulate metabolism. It is quite possible, however, that the effects which are desired might also, in those instances in which no manifest disease of the thyroid is present, be elicited by an adequate increase in the protein intake of the patient. This possibility is merely mentioned in order to illustrate the probable nature of the effects and utility of a protein intake in excess of our minimum needs. We must recollect that it is not the energy-output which suffices merely to maintain life, to gain the means of living for another day, which is of genuine value in the eyes of civilized mankind. The products of human effort which we prize are wholly the outcome of the small surplus of energy which we collectively generate over and above the minimum which will support life and propagate the species. This small surplus, which is minute in comparison with the aggregate expenditure, is the origin of all that we cherish, and, even in purely economical terms, the cost of its production is negligible in comparison with its value. In the absence of any evidence of deleterious influence, a reasonable excess of protein intake, such as that usual in the United Kingdom or America, should not be discouraged in advance of a clear demonstration that it plays no part in the generation of efforts which, in the aggregate, may outweigh the costliness of the practice. It must be admitted, however, that even upon this basis it is difficult to defend the extraordinarily excessive meat-consumption which has hitherto been customary in Australia.

THE NORMAL DIET.

The normal dietary of a variety of different classes and occupations of society in the United States has been investigated by Atwater both from the standpoint of composition and that of **Calorific Value.** The following table summarizes some of his results. It must be recollected, however, that the quantity of food actually digested, assimilated and utilized, was in each instance a little less than the quantity which was ingested.

Occupation.	Composition of the diet.			Calories per day.
	Protein, grams.	Fat, grams.	Carbohydrate, grams.	
Farmers' families	101	128	476	3560
Mechanics' families . . .	113	153	420	3605
Professional families . . .	110	136	442	3530
Five college-student clubs .	127	181	402	3880
Sixteen men's student-clubs .	105	147	465	3705
Four women's student-clubs	101	139	414	3405
Stonemason, hard work . .	180	365	1150	8850
Blacksmith, hard work . .	200	304	365	6905
Footballer	181	292	557	5740
Sandow	244	151	502	4462
Teacher's families, Indiana .	111	110	349	2910
Official's families, Pennsylvania	98	155	396	3465

It will be observed that the habitual performance of hard physical labor is correlated with a high calorific intake. The increase of intake affects, as a rule, all three classes of foodstuffs. The increase of the **Protein** intake is surprising in view of the fact that proteins are not a normal source of muscular energy. This apparent contradiction, which has been observed in all countries, has been explained in several diverse ways. Advocates of a high plane of protein nutrition have advanced the tendency to increased consumption of protein by those who perform hard physical labor, as evidence that the increased speed of metabolism induced by protein facilitates the functional activity of the tissues, including muscular tissues. Advocates of the low plane of protein nutrition, on the contrary, have urged that the high protein intake of these persons is essentially accidental, arising simply from the fact that they ingest larger quantities of all foodstuffs and, maintaining the normal admixture of the three types of food material, incidentally consume more protein. This, however, was certainly not true in the case of the blacksmith and the professional athlete, Sandow, whose dietaries were investigated by Atwater. A more reasonable suggestion than either of the above is probably that which has been put forward by Voit, that as persons accustomed to hard labor are usually more muscular than sedentary individuals, the total protein intake required to support the greater quantity of **Protoplasmic Tissues**, maintaining their wear and tear, and at the same time the exogenous metabolism, is greater than it is in persons, even of like weight, in whom a considerable part of the weight is made up of adipose tissues, for example. The figures obtained by Atwater are certainly suggestive from this point of view, for the total mechanical work performed by Sandow in a brief daily exhibition and a period of practice or exercise was evidently not nearly equal to that performed by a manual laborer in an eight- or ten-hour day. But by exercises and a mode of life carefully directed to that end, Sandow had brought about in himself an extraordinary degree of muscular development, far exceeding that of the ordinary laborer. in harmony with Voit's suggestion we find that his intake of protein was nearly two and a half times the normal, while his intake of fat was normal and his intake of carbohydrates only slightly above the the average.

It has already been pointed out that the vegetable foodstuffs are, as a rule, distinguished by their relatively low content of protein. This arises from the fact that carbohydrates assume a structural rôle in plants while in animals their place as structural materials is taken by proteins. It is from this fact that one of the several objections to the practice of **Vegetarianism** arises. A purely vegetable diet is, if nitrogenous equilibrium is maintained, an exceedingly voluminous one. The indigestible residues of cellulose are large, the feces very bulky, and the fecal masses occlude a proportion of otherwise digestible and assimilable materials which are voided with them. The wastage in a vegetarian diet is for this reason alone a considerable item. A much

more serious source of waste, however, is the incomplete utilizability of the small proportion of protein which the vegetable diet does contain. We have seen from the researches of London (Chapter XI) that the intestinal epithelium exerts a preliminary selective action upon the amino-acids which are submitted to it for absorption, rejecting a proportion of those which are present in unwonted excess. Now the proportions of the various **Amino-acids** in the proteins of vegetable origin differ very decidedly from those which obtain in proteins of animal origin and therefore, on a purely vegetable diet, the amino-acids presented for absorption are in abnormal proportion to one another. A portion of the amino-acids derived from vegetable proteins by digestion are therefore rejected and voided in the feces. The following table shows the percentage of the nitrogen in various types of foodstuffs which is actually assimilated:

Type of food.	Percentage of nitrogen actually assimilated.
Flesh	98
Fish	97
Eggs	95
Milk	94 to 95
Peas, Beans	85
Corn	83
Wheat-flour	81
Rice	80
Potatoes	78

The following shows the relative proportion of wastage on a purely vegetable diet, an average mixed diet and a high meat-diet (Atwater and Langworth):

Type of diet.	Nitrogen in grams per day			Percentage of nitrogen wasted.
	In food.	In urine.	In feces.	
Vegetable diet	13.8	13.9	3.9	28
Mixed, average meat	19.4	15.6	2.4	13
Mixed, large amount of meat	33.1	24.5	2.9	9

Even the amino-acids which fail to undergo assimilation, however, do not represent all the wastage which occurs on a purely vegetable diet, for the process of selection and rejection which initiates in the intestine continues in the tissues, and the rejected excess of unutilizable radicals simply enters the exogenous metabolism and, while it is available for the production of heat, is useless for the maintenance of the integrity and repair or synthesis of tissues. This fact is very well illustrated by the experiments of K. Thomas, who, subsisting upon a diet of starch and sugar, estimated the minimal daily loss of tissue-protein and then added to his diet food materials of various types in order to determine the relative power of the proteins which they contained to save the body from loss of tissue-protein, or, as he terms it, the **Biological Values** of the various proteins. The following were some of his results:

BIOLOGICAL VALUES OF VARIOUS PROTEINS, ESTIMATED IN TERMS OF THE PERCENTAGE OF BODY-PROTEIN WHICH THEIR INGESTION WILL SPARE FROM LOSS.

Ox-meat	104	Yeast	71
Cow's milk	100	Casein	70
Fish	95	Nutrose	69
Rice	88	Spinach	64
Cauliflower	84	Peas	56
Crab-meat	79	Wheat-flour	40
Potatoes	79	Cornmeal	30

It is evident, therefore, that the nutritive value of peas, for example—notwithstanding their remarkably high protein content in comparison with other vegetables—is much less than we might infer from their composition, and approximately double the normal protein intake required on a diet in which peas and beans are the only important source of protein. Recollecting that peas and beans are the only generally available vegetable articles of diet in which proteins are at all abundant, the difficulty of securing nitrogenous and calorific equilibrium upon an exclusively vegetable diet must be apparent. The herbivorous animals can accomplish it by eating an enormous bulk of food, for which their intestines are specially adapted by their length and capacity. A proportionately bulky diet would insure grave digestive disorders in the average human being to whom it was habitual.

Even more serious difficulties than this, however, confront the would-be vegetarian. We have seen (Chapter XX) that certain constituents of the diet which are associated solely with **Animal Fats** are absolutely essential both for maintenance and for growth. These are lacking in a diet composed of customary articles which are solely of vegetable origin. The fat-soluble essentials for growth and maintenance do not occur in the fatty tissues of plants, in seeds and fruits, but in the forage-parts. They are acquired from these by the herbivorous animals and stored by them in their body-fat. To obtain a sufficiency of these substances from vegetables in our diet, we would be compelled to consume an excessive quantity of vegetable material of very low nutritive value, containing a very large proportion of indigestible residue. It may therefore be stated, and experience seems to have fully justified this deduction, that continued maintenance of weight and health, and, above all, growth, are impossible of attainment by human beings who confine themselves strictly to a vegetable diet.

Happily there are few people who are so fanatical in their vegetarianism as to attempt to subsist solely upon vegetables, fruits and cereals, and the so-called vegetarian usually partakes fairly freely of **Milk** and **Eggs**. On a mixed diet which contains a good proportion of these articles there is no difficulty in securing a thoroughly satisfactory nitrogenous and calorific equilibrium, and experience has demonstrated that a dietary of this character may maintain a high standard of bodily health and vigor. It is not improbable that occasional indi-

viduals would positively benefit by adopting a dietary of this type. Others, again, might not improbably find that while it fully sufficed for the maintenance of weight and health and the satisfaction of the appetite, yet better digestion and improved well-being would be attained on a dietary containing some proportion of meat. To the majority, appetite, taste and habit apart, it would probably be indifferent which alternative was adopted. Without positively encouraging such dietetic experiments, especially where children are concerned, the physician will probably, unless there are certain indications to the contrary, do well to allow a vegetarian of this type to indulge his whim. The absolute vegetarian, however, who declines even to partake of milk or eggs, must be solemnly warned of the danger he is incurring and the almost inevitably unhappy outcome of his fanaticism, while his children should be shielded, if possible, from the outrage of the perpetration of his delusion, and irreparable detriment of their bodily welfare.

On the other hand an exclusive flesh-diet, which has been advocated no less warmly than vegetarianism in certain ill-informed quarters, is only a shade less undesirable than an exclusively vegetable diet. The wastage again becomes very large on account of the stimulation of metabolism resulting from the high plane of protein intake, and an abnormally large consumption of food becomes necessary to maintain nitrogenous and calorific equilibrium. The insufficiency of the carbohydrate intake provides little of the proper nutriment for the muscular tissues, the power of continued exertion is impaired, and the tendency to certain types of auto-intoxication is probably enhanced. The diet is so completely digestible that the fecal bulk is too small to maintain the proper tension and tonus of the lower intestine, and the resultant stasis favors **Intestinal Putrefaction.** The abundant variety of mineral constituents contained in the vegetable items of the customary dietary is replaced by the relatively limited variety and quantity of mineral constituents in flesh. The high protein intake implies a high sulphur intake, and therefore the formation of large quantities of sulphuric acid, which reduce the alkali-reserve and impose a tendency toward **Acidosis.**

On the whole, it must be evident from the above discussion that the only safe prescription for continued employment by persons of all ages is that which the good housekeeper instinctively recommends, namely an abundant and *varied* diet. The requirements of the body are so numerous and so varied in their character and in the sources from which they must be derived that in our present state of knowledge a dragnet policy of sweeping into the body a large variety of dietary articles, is the only one which will ultimately ensure a sufficient intake of every possible requisite. All precise dietary prescriptions, however well supported by selected individual experiences, are premature where the majority of humanity are concerned, and a diet of half-raw meat, recommended on the ground that, being muscle, it must contribute to our strength, should be viewed with no less suspicion than a diet of

nuts, advocated because some of our arboreal ancestors were perforce accustomed to partake of these indigestible delicacies rather freely.

The physician, of course, will find it imperative from time to time to impose quite severe restrictions upon the dietary of certain types of patient, of diabetics, for example, or of persons afflicted with nephritis, or with certain types of indigestion, and often he will achieve very great success by this simple means. His very success, however, constitutes in certain cases a positive danger to other people, through the possible conversion of his patient into a dietary propagandist seeking to promulgate a "system" arising out of the measures which were found effective in bringing about the recovery of his own health. A brief but clear and simple statement by the physician of the precise object of the dietary imposed, and its limited applicability, might, not infrequently, suffice to stifle a dietetic fad at its birth.

THE CALORIFIC REQUIREMENT AND THE "SURFACE-LAW."

The average **Starvation-metabolism** of a vigorous man engaged in light work and weighing 70 kilos is about 2240 calories or 32 calories per kilo. To maintain calorific equilibrium this heat-value must be contained in the food, and a certain excess to compensate for the stimulation of metabolism or **Specific Dynamic Action** of foodstuffs. On a normal mixed diet this amounts to from 11.1 to 14.4 per cent. of the starvation-minimum (Rubner). This would indicate calorific equilibrium on an intake of from 2488 to 2562 or, in round numbers, 2500 calories or 36 calories per kilo of body-weight.

The total metabolism varies very greatly in different species of animals, the metabolism per kilo being much higher in small animals than in large. This may be inferred from the relative consumption of **Oxygen** per hour and kilo body-weight by different species. The following results are cited after Rubner:

Species.	Weight, kilos.	Grams of oxygen consumed per kilo per hour.
Calf	115.	0.481
Sheep	66.	0.490
Turkey	6.2	0.702
Dog	5.6	0.902
Goose	4.6	0.677
Rabbit	3.43	0.735
Hen	1.51	0.846
Duck	1.22	1.382
Finch	0.025	13.000
Sparrow	0.022	9.595

The greater metabolism of the smaller animals arises, according to Rubner and Richet, from the greater area of external surface in proportion to their volume which they present. If the linear dimensions of a solid are increased in the proportion of 1 : 2 the surface is increased in the proportion of 1 : 4, but the volume in the proportion of 1 : 8, so that the ratio of surface to volume falls to one-half. The surface of

a regular solid varies as the two-thirds exponent of the volume or as $W^{\frac{2}{3}}$, if we measure volume in terms of weight. Now Rubner has observed that the metabolism per unit of **Body-surface** is much more uniform in different species than the metabolism per unit of body-weight. E. Voit has determined the heat-production in resting animals of various sizes per kilo and also per square-meter of surface with the following results:

Species.	Weight in kilos.	Calories produced. Per kilo.	Calories produced. Per sq. M. surface.
Horse	441.	11.3	948
Pig	128.	19.1	1078
Man	64.3	32.1	1042
Dog	15.2	51.5	1039
Rabbit	2.3	75.1	776
Rabbit (without ears)	2.3	75.1	917
Goose	3.5	66.7	969
Fowl	2.0	71.0	943
Mouse	0.018	212.0	1188

The metabolism per kilo in these different species displays the greatest diversity, ranging from 11 calories per kilo in the horse to 212 calories per kilo in the mouse. The metabolism per square-meter of body-surface is very nearly the same in all of the different species investigated, ranging, with the exception of the rabbit, from 900 to 1200 calories per square-meter. Metabolism bears therefore a far closer relationship to surface than it does to weight and the relationship extends to different individuals of the same species and explains in part the high metabolism of infants.

This relationship, which was discovered by Rubner in 1883 and emphasized by Richet in 1885, was at first interpreted to mean that the main factor governing metabolism was the rate of **Radiation of Heat** from the surface of the body. Doubt was thrown upon this interpretation, however, by the discovery that the production of heat by warm-blooded animals of different sizes continues to be proportional to the body-surface even when the temperature of the surroundings is uniform or nearly uniform with that of the body, so that the heat-loss through radiation is a negligible proportion of the total energy-production. On referring to the preceding table it will be noted that in a rabbit deprived of its ears, although the radiating surface is much diminished, yet the production of heat remains unaltered.

Although the metabolism per unit of surface varies very much less than the metabolism per unit of weight, yet the proportionality of metabolism to surface-area is not nearly so exact as many observers have in the past decades considered it to be. Thus Benedict, in a critical examination of the ratio of **Basal Metabolism** to surface in eighty-nine men, sixty-eight women and a large number of infants found very marked deviations from the rate of strict proportionality. As Benedict has stated: "It is obvious that any basis of comparison which involves variations of 40 per cent. with men, of 43 per cent. with women, and

80 per cent. with normal infants, cannot be considered as a physiological law." Benedict draws attention to the great importance of specific **Stimulators of Metabolism,** which may be contained in the diet or in the products of the activity of certain tissues. Thus after prolonged severe **Muscular Exertion** the metabolism is stimulated for a long period following the cessation of exercise and the consumption of foodstuffs for the production of mechanical work. Yet the ratio of bodily surface to volume has undergone no change in consequence of the exercise, nor has the temperature of the body risen. Whatever may be the mechanism which brings it about it is clear that products of muscular exercise (and the same is true of acidosis) induce a stimulated combustion of foodstuffs, and therefore, in the absence of ingested food, an increased destruction of tissue.

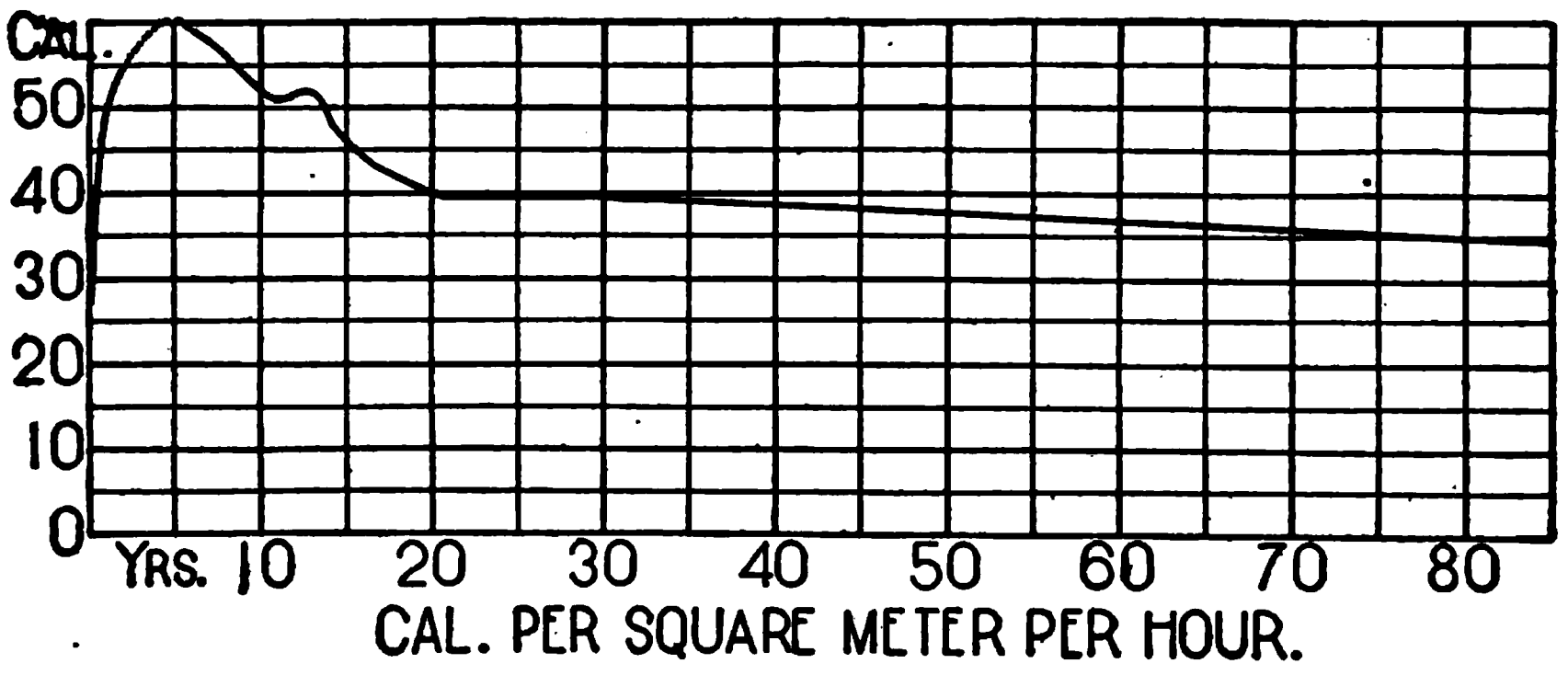

FIG. 50.—Chart, prepared by Du Bois, showing the basal metabolism as measured in calories produced per square meter of body-surface per hour from birth until the age of eighty-five years in human males. Between maturity and the eighty-fifth year there is a gradual fall in the intensity of metabolism of 13 per cent. (After Lusk.)

Benedict infers that the total metabolism, or metabolism at rest without food, is determined by two main factors; the first the mass of **Protoplasmic Tissues** (parenchyma) and the second the variable concentration of specific **Stimulators of Metabolism** in the tissues. It was, in fact, assumed by Voit that the total metabolism is actually proportional to the mass of cellular as distinguished from **Sclerous Tissues** in the body and this view is supported by the steady decrease in metabolism which is characteristic of the period between maturity and old age in man (Fig. 50). The *increase* in basal metabolism per unit of weight or surface which occurs to a very striking degree during the first year of post-natal growth is, however, only to be interpreted by also taking into consideration the second factor suggested by Benedict, namely the variable concentration of stimulators of metabolism which determines the **Metabolic Rate** of the tissues. The rise in metabolism which occurs in early growth and just before puberty, therefore, indicates an accumulation of stimulators of metabolism which are not improbably the **Endogenous Catalyzers** of growth.

We must still admit that the ratio of basal metabolism to surface, although variable, is much less variable than the ratio of metabolism to weight, length, temperature, or any other dimension or characteristic of the individual. The possibility has not been sufficiently considered, however, that many details of structural proportion in the body may be correlated with superficial area rather than with weight, and that the observed relationship of metabolism to surface may be thus only an indirect one, representing a relationship of metabolism to a group of structural elements which vary as the two-thirds exponent of the body-weight or volume. Thus Dreyer has shown that the blood-volume and the sectional areas of the aorta and the trachea of animals of different size are proportional to $W^{\frac{2}{3}}$, that is, to the surface. Friedenthal has pointed out that the sum of the non-protoplasmic materials (reserve-materials, skeletal constituents and fibrous tissues) in the animal body increases more rapidly with total size than the protoplasmic tissues. This is, in fact, inevitable, for the need of binding and supporting tissues increases in proportion to the strains to which the body is subject and these increase not only in proportion to the mass but to the mass $\times$ linear dimensions of the body. A small mass of protoplasm requires no binding tissues to support it, but a large mass of cells would collapse of their own weight without binding, cementing and supporting tissues, and the greater the distance of any mass of protoplasm from the center of gravity of the whole, the greater in that proportion will be its tendency to break away. Friedenthal concludes, in fact, that the protoplasmic or **Parenchymatous Tissues** only increase in proportion to the two-thirds exponent of the total weight, *i. e.*, in proportion to the surface. Since these are the tissues of highest metabolic rate, their mass, together with the proportion of **Endogenous Catalyzers** which they contain, might be expected to play a leading part in determining the rate of basal metabolism.

THE NUTRITION OF CHILDREN.

During the early period of post-natal development the sole normal source of food among the mammalia is **Milk**. The milk of different species of animals, however, is very far from being of constant composition, and we may infer that the optimal admixture of foodstuffs for sucklings varies greatly with the species. The following table represents the composition of milk of several species, determined by Abderhalden.

One hundred parts by weight of milk contain:

Species.	Casein.	Albumin.	Total protein.	Fat.	Sugar.
Dog	4.8	2.6	7.4	11.6	3.2
Pig	3.8	1.5	5.2	9.5	3.3
Sheep	4.1	0.8	4.9	9.3	5.1
Goat	2.9	0.8	3.7	4.3	3.6
Guinea-pig	4.8	0.6	5.4	7.0	2.0
Cow	2.9	0.5	3.4	3.7	5.0
Horse	1.3	0.8	2.1	1.1	5.9
Ass	0.8	1.1	1.9	1.4	6.2
Human	0.8	1.2	2.0	3.7	6.4

Human milk contains more **Albumin** and much less **Casein** than cow's milk. This may be only one among many reasons, not readily determinable by analysis, why **Artificially-fed Infants** rarely thrive as well as breast-fed infants. This fact, which has so often been demonstrated and in such a diversity of ways, may be illustrated by the following tabular comparison of the growth of South Australian male infants which were in every respect normal, but which in the one group were fed for at least the first few weeks at the breast, while in the other group modified cow's milk was the source of nutriment:

	Average weight in ounces of South Australian male infants.	
Age in months.	Breast-fed.	Bottle-fed.
1	155	117
2	187	141
3	206	169
4	224	193
5	254	226
6	270	242
7	287	267
9	311	280

The nutritional requirements of children are much greater in proportion to their weight than those of adults. The heat-production of infants at various ages is thus summarized by Murlin:

	Heat production of infants recently fed and sleeping.	
Age.	Calories per kilo and hour.	Calories per square-meter of surface and hour.
Birth	1.87	25
2 to 4 months	2.38	35
6 to 12 months	2.45	42

Underfed and atrophic infants produced more, and overweight infants less than the heat-output of normal infants. It must be remembered, however, that these figures are subject to considerable modification by a variety of factors, among which **Exercise**, for example crying, the type and quantity of **Clothing** worn and the **Temperature** of the surrounding atmosphere are the most important.

The **Heat-production** per kilo body-weight in an infant during the first year is about 80 calories, while that of an adult does not exceed 36 calories per kilo. The heat-production of the **Newborn Infant** is much less than at later months, in many cases not exceeding 48 calories per kilo. The heat-production per square-meter of surface also rises during the first year. The allowance of 100 calories per kilo which is adopted by many physicians upon the basis of the older estimations of Heubner is undoubtedly excessive for the average infant. Even taking 80 calories per kilo as a basis, however, the food required by an infant of 10 kilos at one year of age is one-third of that required by an adult weighing seven times as much.

This high food-requirement arises from three sources: Firstly the high average **Metabolic Rate** and the high proportion of **Parenchymatous**

Tissues in young animals, secondly the larger proportion of surface to volume involving a greater **Radiation of Heat** than in the adult, and thirdly the energy absorbed in the building up and retention of new tissue. In older children we must add to these the incessant **Muscular Activity** which characterizes a healthy child. Taylor states that a resting boy of ten years should have a metabolism of about 40 calories per kilo per day, but when engaged in play the diet of the child may have to be as high as 100 calories per kilo per day to maintain calorific equilibrium. "The diet of a child must, therefore, cover the basal metabolism, the natural increment of growth, and the enormous output for physical exercise. It is the inability to judge these fractions correctly that is responsible for so much underfeeding of children. There are furthermore the additional deprivations so often inflicted on children by the application of fad-notions of diet. The relative caloric input of a normal child leading an outdoor life is to be compared to that of a man at heaviest physical work. Protein in excess is not needed, that is clear; but total calories are needed, in the form of sugar and fat." The craving of healthy children for sugar is therefore the expression of a normal and healthy need arising from the high consumption of glucose by the **Muscular Tissues**. It should be satisfied by a discreet allowance of sugar and an abundant allowance of polysaccharides.

THE ENERGY-EQUIVALENT OF GROWTH.

The storing-up of tissue-substance which is possessed of a definite calorific value, necessarily results in the retention by the growing animal of a proportion of the energy-value of its food, and, furthermore, a considerable proportion of the heat-value of the diet, varying with age and the rate of growth, is additionally consumed and dissipated in performing the work of storage. This is doubtless attributable to the fact that at all stages of growth, as at all stages of any chemical transformation, the forward and reverse reactions are proceeding side by side. In growth the products of the reverse reaction (tissue-degradation) participate in a side-reaction (**Exogenous Metabolism**) and are thus partially consumed and their energy-value dissipated in the form of heat, mechanical work, and the energy-values of the excreta. Hence we find that in a given species of animal, the slower the accretion of tissue the greater the energy consumed per kilo of tissue built up, since the reverse reaction in such a case is proceeding for a longer time. The following are illustrative results obtained by Aron:

GROWTH OF DOGS.

Animal number.	Calories consumed in fifty days.	Increase in weight in fifty days.	Calories consumed per gram of tissue-increase.
B	19,950	1570	12.7
C	13,925	1000	13.9
VIII	9,500	780	16.4
XII	10,750	838	15.6

From these results it is clear that an animal gaining 1000 grams in fifty days needs fewer calories for this gain than one gaining 1000 grams in one hundred days, the reason being, as indicated above, that in the former instance the animal needs to be "maintained" for only one-half-as long as the latter. The following are comparable observations made by Aron upon Filipino children:

MARIA INOCENCIA.

From week.	To week.	Number of days.	Increase in grams. From	to	Per day.	Calories. Per day.	Per kilo.
21	26	35	3500	3600	3	350 to 375	100 to 105
26	31	31	3650	4225	17	450	115 to 120
31	35	28	4225	4811	21	500	125

MIGUELO PRIEGA.

From week.	To week.	Number of days.	Increase in grams. From	to	Per day.	Calories. Per day.	Per kilo.
4	9	35	3550	4175	17	350 to 400	100
9	13	28	4175	4850	24	450 to 475	105

Hence, during the entire period of the investigation, Maria Inocencia increased in weight at the rate of 14 grams per day and consumed an average of 450 calories per day and 115 calories per kilo. Miguelo Priega, on the other hand, increased in weight at the rate of 21 grams per day and consumed about the same number of calories per day and a considerably smaller number per kilo.

According to Rubner, the energy consumed per kilo in doubling the **Birth-weight** of animals is always very nearly the same, excepting in the case of man, namely about 4000 calories. The following data are presented by Rubner in support of this thesis:

Species.	Energy-consumption per kilo in doubling the birth-weight.
Horse	4512
Cow	4243
Sheep	3926
Hog	3754
Dog	4304
Cat	4554
Rabbit	5066
Man	28864

The generalized form of this relationship would be:

$$\frac{E}{x} = a \log x + b$$

where "E" is the energy-consumption, "x" the weight of the animal and "a" and "b" are constants which are the same for all species (excepting man). Doubling of the weight would obviously always add an equal amount to the quotient $\frac{E}{x}$, that is, to the total energy-consumption per kilo. This leads to the differential or velocity-equation:

$$\frac{dE}{dx} = \frac{E}{x} + a$$

which means that the consumption of energy per unit of tissue-accretion increases in proportion to the energy which has already been consumed in reaching the weight to which this unit of tissue is added. When this rate of energy-consumption becomes equal to or less than the **Basal** or **Maintenance-metabolism** it is obvious that growth must cease. The more rapidly growth occurs, however, the less energy derived from exogenous metabolism is expended during the time consumed in building up a unit of tissue at a slower rate. This obviously corresponds very well with the facts ascertained by Aron.

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THE OUTLOOK.

The acquisition of knowledge always results in the revelation of wider and yet wider prospects tempting inquiry and inviting exploration. To the Pythagoreans life and the universe were fairly simple, a few rules when once discovered would, they felt sure, reduce the seeming chaos to order. In the laws of number lay the simple clue to the whole riddle. To Descartes, two thousand years later in the history of man and of science, how much more complex did the world appear. Yet even he thought that the phenomena of life could be interpreted by geometry and hydrostatics and that emotions arose through oscillations of the Pineal gland, originating from the varying pressures of an impinging fluid. But three-quarters of a century later, Newton, incomparably the greatest discoverer of his age, gazed in awe and humility upon the limitless prospect which his labors had revealed: "I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, while the great ocean of truth lay all undiscovered before me." A new ocean of undiscovered truth:—that is the revelation which we glean from every fresh achievement of the scientific method, and this is essentially its most inspiring outcome.

It is refreshing, from time to time, to pause amid the fruits of our collective labors and gaze upon the widened prospect which lies before us, striving to make out the dim form of truths which are emerging, half-veiled in the mists of the early dawn of knowledge, upon the horizon of our inquiries.

In the territory with which we are here most particularly concerned; that territory which lies upon the borderland of life and of atomic affinities, and seeks to illumine the one with the beacon-lights of the other; the unexplored oppresses us with its vastness and entices us with its promise, while the known, the sure ground of fact, comprises only the fringe of our future heritage of knowledge. In the prospect which lies before us certain objectives lie plainly outlined and almost within our grasp, others are less clearly apprehended and others, again, loom gigantic, unformed, terrible in their potentialities for good or for evil, upon the ultimate horizon of our outlook.

In the forefront of our prospect lie, patently enough, the vast industrial potentialities of our science, barely touched as yet, but destined in the near future to be a rich field of endeavor, promising inexhaustible resources of wealth and power, the physical foundations of intellectual achievement. The accumulated storehouses of fuel,

deposited in the carboniferous era, and now rendered available to us in the form of coal and oil, have merely served, by one of those happy conjunctions of historical circumstance which have rendered possible the spiritual development of man, to tide us over the period of awakening consciousness and undeveloped powers which comprised the age of steam and the industrial revolution of the nineteenth century. Within a period which is relatively brief in the age-long history of man these stores will be exhausted and we must, as we assuredly will, long ere that term arrives, solve the problem of manufacturing illimitable supplies of fuel. Ultimately there is only one way in which this can be done, and that is by transforming the radiant energy of the sun into the potential energy of a falling weight, originally lifted by the heat absorbed in evaporation, or else, as in the utilization of alcohol for motor-fuel, by converting the radiant energy of the sun into the potential chemical energy of a carbohydrate or a related or derived organic compound. The latter method lies almost within our control, the former not so nearly, and hence it is to the understanding and control of the photochemical synthesis of organic compounds that we must look in the main for our future sources of fuel and motive-power.

The initial step of photosynthesis having been accomplished, the succeeding stages in the evolution of organic compounds in living organisms are accomplished at low temperatures through the agency of enzymes. We are gathering acquaintance with the nature of these substances and of the circumstances and principles which govern their action, and through their right understanding and employment we will ultimately be enabled to accomplish syntheses which at present are possible only in living organisms or, if imitable in the laboratory may only be achieved at the cost of an expenditure of energy and raw materials far exceeding the value of the product.

The further investigation of the oxidative processes which occur in living organisms and underlie luminescence, is undoubtedly destined to supply us with that hitherto elusive ideal, "cold light," and the remarkable advances in our knowledge of this field during the past few years, assure us that this outcome of biochemical investigation is not now very far from practical realization. The meteoric advance of aviation, from the air-flotation experiments of Langley to the recent flight from London to Australia, has shown us how rapidly in our times practical realization may follow upon the heels of theoretical possibility.

The fuller understanding of the nature of enzymatic processes which lies immediately before us will, ere long, lead to the discovery of their chemical nature and composition. Advances have already been made in this direction. Euler has produced an artificial oxidase, and Falk an artificial lipase. It is not at all improbable that the digestive enzymes are not nearly so complex as the earlier investigators imagined and that the synthesizing enzymes are merely the digestive enzymes or modifications of them, acting under differing physical conditions.

The synthesis and control of artificial enzymes will revolutionize the science and art of organic synthesis and place in our hands a multitude of inestimably valuable products which have hitherto been regarded as costly rarities, the curiosities of a chemical museum. At the same time, of course, the production of many substances which are already manufactured, or derived from the cultivation of plants or animals, will be very greatly cheapened. But, above all, the artificial production and the control of enzymes holds out the hope of accomplishing the synthesis of foodstuffs under conditions independent of climatic variations, and in the immediate neighborhood of the great centers of population, thus eliminating for the great majority of humanity the enormous addition to the cost of food-values which is comprised in the expense of transportation. The synthesis of palatable carbohydrates and fats, sufficing for a certain proportion of our dietary, when we once acquire control of the enzymes, should not present any insuperable difficulties. The proteins are a far more complex problem because of the diversity of units of which they are composed and the necessity for the provision of each one of them, nor will the synthesis of amino-acids suffice, for while these satisfy merely nutritional requirements they are not palatable and their ingestion in requisite amounts introduces abnormal conditions into the alimentary canal which are not well tolerated. The synthesis of "protein-sparers" of the type of gelatin, polypeptides which may be utilized with advantage to reduce our protein ration, would doubtless be the first step in this direction.

After, all, however, it may well turn out that the most practicable way to synthesize enzymes is to permit organisms to make them for us. Not the complex organisms of the present-day farm, but unicellular organisms which we may cultivate in vats. We have utilized such organisms since the earliest dawn of history to make alcohol and acetic acid for us, and at the present day we utilize unicellular organisms, yeasts or bacteria, in the manufacture of bread, of cheese, in the preparation of hides for tanning and other processes of manufacture. This type of industry, which is as yet barely in its infancy, has received a powerful stimulus through the necessities created by the war, and while in the allied countries a special organism was utilized to manufacture acetone for the preparation of explosives, in Germany yeast was cultivated in media consisting of inorganic salts and glucose, as a means of manufacturing protein. This protein, and the fats, polysaccharides (glycogen) and vitamines which the yeast-cell also contains, might well be employed as a desirable and palatable article for human consumption, but the method in which it was chiefly employed in Germany during the war appears to have been as a concentrated feed, economical of production and transport, for the nourishment of cattle.

The gradual replacement of the crude and wasteful, but picturesque and health-giving processes of the farm, interwoven with our remotest origins and endeared to us by innumerable historial associations, by

the "sordid" processes of the factory may well seem to many a far from desirable outcome. The scientific investigator, however, like the follower of a religious order, stays not to inquire whether this or that particular consequence of his faith be immediately good or bad in its transient outcome. We cling to the faith that the comprehension of nature will yield ultimate fruits of unalloyed good. The forward march of that comprehension cannot be stayed for the loss of this or that implement of our intellectual youth which must, albeit with poignant regret, be discarded by the way. The ultimate triumph of spiritual over material interests, values, and motives, which is the goal of our understanding, will yield us pleasures upon another plane, as incomprehensible to us, perhaps, as ours are to the primitive savage. Furthermore if the factory is "sordid," that is, after all, not the fault of the knowledge that rendered manufacture possible, but of the decrepit ideals and stunted imagination of those who utilize our knowledge. The social evils which menace civilization in our day are the indirect outcome it is true of the advances of scientific knowledge, but the responsibility for them rests upon the whole of humanity; they are the visible expression of defective ideals, defective understandings and defective information; they are not of the essence of knowledge, nor does the guilt of their production oppress the soul of the pure seeker after knowledge. An ape knows not how to use fire nor the savage how to use edged tools. Both may hurt themselves with these things, but does it follow then that they are bad or that knowledge of them should be eschewed?

Perhaps, after all, the substitution of the factory for the farm may restore, rather than detract from the value of the country to man. Regret it as we may, and long before the factory-synthesis of foodstuffs has begun to be a measurable item in our commerce, the attractivenesss of agriculture as a career is diminishing and has already fallen far below its ancient standard. The restoration of our countryside to untamed nature may serve us after all in good stead, and set free for us the means of enjoying some of the pleasures of primitive man once more, of regaining some of the youth of the world with the intellectual heritage and the securities of an old and complex civilization.

Returning, for the moment, to more immediately realizable possibilities, the utilization of the various products and constituents of living matter, apart from the foodstuffs, is as yet in its infancy. The value of materials arises out of their peculiar suitability for the purposes of man, on the one hand, and their rarity on the other, and the desires and purposes of man are so multifarious in their variety that it may be said that any material possessed of unique physical characteristics will ultimately be found of peculiar utility in satisfying some one or other of our needs. Now among the products of vegetable and animal life, there are numerous substances which are distinguished by their possession of unique physical characteristics. The peculiar properties

of rubber and of the gums and mucilages, the adhesive quality of gelatin, the glaze communicated to surfaces by colloids in general and starch and dextrins in particular, and the hard surfaces communicated by the drying oils are already utilized in a multitude of ways in our manufactures and our daily affairs; but the possibilities held out by the products of life are far from being exhausted by these few instances. Among the proteins, for example we find elastin, distinguished by its possession of the rare combination of elasticity and tensile strength without rigidity, spongin exhibiting, although in a different way, a similar combination of qualities, keratin, distinguished by its hardness, insolubility, translucency and ability to take a polish, fibroin distinguished by its extraordinary tensile strength, lightness and insolubility. These few examples suffice to show us what a variety of physical characteristics the various proteins may display, and since these substances do not differ profoundly from one another in structure and composition, we may infer that a relatively slight chemical change may confer upon a protein an entirely new series of physical characteristics. An example of this is afforded by the effect of union with formaldehyde upon the physical characteristics of casein.

The proteins are, at present, sparingly employed in the manufactures, but casein is used as a substitute for celluloid, and buttons, hair-combs, billiard-balls, and other objects formerly made of ivory or celluloid are now made of casein rendered horny in consistency by treatment with formaldehyde or calcium hydroxide. Casein is furthermore utilized as a vehicle for pigments in paints, as a finishing and water-proofing material, and for the manufacture of non-inflammable moving-picture films. The uses of gelatin are manifold and well-known. The employment of the relatively expensive proteins of animal origin in the manufacturing industries, however, is excessively wasteful and cannot continue indefinitely, or expand to very great dimensions. We must seek substitutes for the proteins already used, and new utilities as well, among derivatives of the relatively inexpensive vegetable proteins. The exigencies of the war have, in fact, already called into being a vegetable glue, and a vegetable substitute for casein undoubtedly merely awaits the seeker.

In agriculture, our recent acquisitions of knowledge in the field of growth have already profoundly influenced our practice in the feeding of stock for the market and for breeding purposes. Further advances in this direction, together with precise knowledge of the time-relations of growth in the various domesticated animals, will ultimately enable us with the utmost precision to define the most economical practice of feeding and the optimal duration of growth for the production of calorific and nitrogenous values. In the growth of perennial crops, also, an exact knowledge of the time-relations of the growth-process will enable us to determine with precision the optimal period of growth which should elapse before cropping. Especially in forestry this knowledge will increase the economy of our practice.

The biochemical relations between the soil and its bacterial flora on the one hand and the crop on the other is already a flourishing field of investigation, and the results of these inquiries have led to very important improvements in agricultural practice. The further development of this field, and especially the expansion of our knowledge of the metabolism and symbiotic relations of bacteria, will point the way to a multitude of new industrial and agricultural applications. The subject of plant-pathology is also intimately related to biochemistry and the investigation of the biochemical conditions underlying gall-formation, for example, will undoubtedly shed a flood of light upon the essential nature of the internal factors which govern the growth of plants.

It is in the practice of medicine, however, that the applications of biochemistry will ultimately come to affect human welfare most directly and profoundly. At the present moment the advances of biochemical knowledge and technique are rapidly furnishing the physician with diagnostic methods of precision, and indications for treatment based upon exact knowledge, where but a few years ago empiricism afforded the sole basis of treatment. The discoveries which lie before us, however, will ultimately transform the scope, and revolutionize the practice of medicine, and the substitution of knowledge for empiricism, of science for craftsmanship, as yet barely begun, will not cease until it is complete. The life of man may be regarded from a material point of view as consisting on the one hand of a struggle to obtain nutriment, clothes, and other essentials of existence, and on the other hand a struggle to withstand the deleterious influences of his environment and the imperfections of his own organization. Our environment opposes us with climatic fluctuations and extremes, and with pervading toxic agents, and an ever-present host of parasitic organisms continually seeking, and barely failing in the conquest of our tissues. On the other hand we display the imperfection of our organization in disorders of function and in the culminating disorder of senescent atrophy.

Each of these disabilities we are seeking to conquer and in their conquest and control biochemistry must necessarily play a leading if not an absolutely decisive part. Our resistance to toxic agents of environmental or endogenous origin is rendered possible by a peculiar mechanism of adaptation, or "tolerance," which we as yet understand very imperfectly. Its understanding and control will constitute one of the most important among the forthcoming advances of our knowledge, and must result, not only in a greatly improved knowledge of the fundamental mechanisms of adaptation, but in throwing a flood of light upon pharmacological science and therapeutic practice. The advances of recent years have demonstrated to us that our resistance to the invasion of parasites is determined by specific chemical agents which our tissues manufacture—the various antibodies. The chemical nature of these substances is as yet hardly understood at all, yet this

knowledge is fundamental to our control of zymotic diseases. We find that whereas to certain organisms we oppose an impenetrable resistance, to others our resistance is very slight. Our acquired resistance, resulting from infection or artificial immunization, varies between the same extremes. The transient or inappreciable immunity conferred by immunization in many diseases lays us open continually to their inroads with resulting loss of life and efficiency which have been displayed upon a gigantic scale in the recent world-wide scourge of influenza. The erection of defenses against such plagues, and the common infections of the respiratory or alimentary tracts which are responsible, in the aggregate, for so much loss of effort, time, life and efficiency in the world, will never be possible until we understand the underlying chemical reasons why resistance, natural or acquired, to this disease should be high and permanent and to that, slight and transient, and our understanding of this will in turn depend upon the acquirement of knowledge of the actual chemical nature of the antibodies and the precise nature of the processes involved in their interaction with the tissues or toxins of the invading parasite. The study of these substances and reactions is proceeding apace, and a clear and full understanding of the mechanisms of immunity, while perhaps as yet remote, will unquestionably be acquired.

The conquest of zymotic disease has begun, many of the bitterest scourges of the middle ages have disappeared from our lives never to return, and one by one our parasitic enemies are being deprived of power to mar or destroy our lives. Our disorders of function are gradually becoming understood, chlorosis and gout are disappearing, myxedema may be prevented, such conditions as cretinism and asthma are being traced to avoidable origins, diabetes is coming under control, and while cancer still exercises its ravages almost uncurbed that dark problem too now presents some openings which the forthcoming advances of our knowledge of the chemistry of growth will undoubtedly enable us to convert into means of its eradication or prevention; for the problems of pathological growth are fundamentally identical with the problems of normal growth, and the information which sheds light upon the one type of growth will reveal the origin of the other.

Senescence alone remains untouched, the final triumph of nature over the human desire to live; but if we can once rid ourselves of the suggestive influence of age-long experience and view the phenomenon impersonally, as the culmination of a definite, understandable and therefore controllable process, we will perceive that this too must ultimately fall under the sway of human intellect. The indefinite prolongation of his own life is the manifest destiny of man, and the progress already achieved is certainly not less than that which had been made toward our conquest of the air when Leonardo da Vinci so confidently, and as it then seemed so futilely, predicted that man would ultimately fly.

The goal of the biological sciences has been stated by J. Loeb to be the artificial creation of living matter. To this, too, we dare not ascribe impossibility, but its attainment seems at present to be almost certainly more distant than any of the objectives we have hitherto reviewed; for our increasing knowledge of life-phenomena reveals to us more and more clearly that the processes of life are wrapped up, not merely with a peculiar admixture of unstable chemical compounds, but also with a definite architectural arrangement of these compounds. The simplest living organism with which we are acquainted possesses a definite structure, and even supposing our knowledge of the chemistry of life to have become so exhaustive as to permit the precise imitation of the chemical constitution of living matter, its structural constitution would still remain an incentive to investigation and an obstacle, but not an insuperable one, to the attainment of our ultimate goal.

The slow, hesitating, clinging grasp of science, like that of the many-tentacled denizens of the sea, cannot be loosened or evaded. Through many trials and failures, let the superficial appearance which hides the precious truth be as polished and impenetrable-seeming as it may, a flaw will be found, a foothold gained, and atom by atom, through centuries if need be, the very heart of mystery is unveiled. There is not, nor ever can be in our universe, anything which directly or indirectly can be made to assail the senses of man, that his intellect cannot ultimately fit into the supreme architecture of the mind, and there is not, nor ever can be, one thing which the intellect of man fully comprehends which he cannot in some measure appropriate and employ for the direction of his own destinies. But in what way will we employ these powers? That, indeed, is a riddle to which science can furnish no solution; its answer lies hidden from our senses, in the deepest recesses of the moral nature of man; but the responsibility for the choice, whatever it may be, rests not with the scientific discoverer, save only in the degree to which he shares our common humanity.

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